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THE NATURE OF THE LIQUID STATE

THE characteristic properties of the liquid state may be grouped under various headings, the most familiar being the mechanical properties, *viz.*, the capacity to transmit hydrostatic pressure, the viscous resistance to flow and the tension exhibited by free surfaces. The elucidation of these and other physico-chemical properties of liquids in terms of their molecular structure is one of the major problems of physics to which a great deal of attention has been given. Laplace's theory of capillarity, and Van der Waal's equation of state are the classic examples of attempts to explain liquid behaviour. They are typical of the purely theoretical approaches to the subject in which hypothetical assumptions regarding the properties of molecules or the nature of the liquid state form the starting point, while the resemblance between the conclusions drawn from the theory and the experimental facts of observation is regarded as an indication of the truth of such assumptions.

That a wholly different approach to the problems of the liquid state is possible became apparent from the investigations undertaken by the present writer in 1921 in order to explain the dark blue colour of transparent oceanic waters. Observations made during sea voyages in that year suggested that this phenomenon had its origin in the molecular diffusion of light in great depths of clear water traversed by sunlight. This explanation was confirmed by laboratory observations which showed that dust-free water traversed by a beam of light exhibits a feeble blue opalescence. The intensity of this opalescence was found to be in fair accord with a formula originally

developed by Einstein from the thermodynamic theory of density fluctuations to explain the enormously stronger effect observed in fluids at the critical temperature. It soon became clear, however, that Einstein's theory did not cover all the facts, and that the diffusion of light in liquids was in reality a molecular phenomenon, and that its detailed study could be expected greatly to enlarge our knowledge of the liquid state of molecular aggregation.

As is well known, a substance may exist in different physical states, *e.g.*, a gas or a vapour, a liquid, an amorphous glass or a crystalline solid. One of the most important questions regarding the liquid state is that of its relation to the other possible states of molecular aggregation. The comparative study of the molecular diffusion of light in the different physical states of a substance is very helpful in enabling the nature of the differences between these states to be understood. The most readily observed changes are those of the *intensity* of the diffused light. This is a maximum in the gaseous state when considered in relation to its density, and a minimum in the crystalline condition, while the liquid and the amorphous solid usually occupy the intermediate positions in the order stated. The scattering of light, though fairly intense and therefore easily observed in liquids, is usually much feebler than the scattering in the vapour if the far greater density of the liquid is taken into consideration. These facts stand explained when it is recalled that the molecules in a vapour are distributed in space at random and in a crystal with geometric regularity, while they indicate that the space-grouping in the liquid and in the amorphous solid is very far from being chaotic, though it does not possess the complete regularity characteristic of crystals. If we accept the thermodynamic theory of Einstein, the compressibility of the substance is, in each case, a measure of the spatial uniformity of distribution. The smaller the compressibility, the more nearly perfect would be the uniformity of the space-distribution of the molecules, and the more completely, therefore, would the secondary radiations from the molecules cancel each other out by interference.

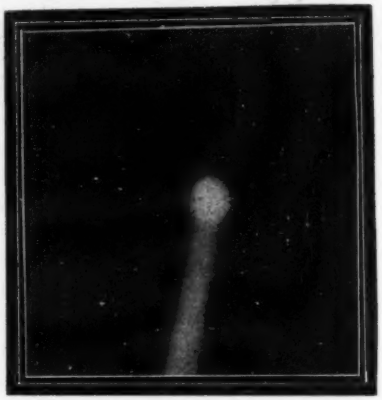

Fig. 1

Volume and surface scattering of light in liquid methyl alcohol
(Photograph by P. S. Hariharan)

We have next to consider the orientations of the molecules in a liquid. In the crystal, as we know, these orientations form a geometrically regular pattern and taken together with the optical anisotropy of the molecules determine the optical birefringence of the crystal. Since liquids are optically isotropic, it follows that there can be no regularity of molecular orientation when averaged over any volume of

macroscopic dimensions. We are justified, however, in asking whether there is any tendency for neighbouring molecules to align themselves similarly or to form groups in which near neighbours stand in some definite relationship. Comparative studies of the intensity and the state of polarisation of the light scattered transversely in vapours and in the corresponding liquids furnish valuable evidence on this point. The optical anisotropy of the molecules in a fluid or amorphous solid gives rise to an "orientation" scattering which is depolarised to the extent of 6/7, and is therefore readily distinguished from the fully polarised "density" scattering associated with the isotropic part of the polarisability of the molecules. It is readily shown that if groups of neighbouring molecules in a liquid were similarly orientated, the orientation scattering would be enormously enhanced in its intensity as compared with its intensity for a state of completely random orientation. Actually, we find that the depolarisation exhibited by the light diffused within a liquid is usually much more striking than the depolarisation of the light scattered by the vapour. This cannot, however, be regarded as indicating that the orientation scattering is larger in the liquid than in the vapour when considered in relation to the density. Indeed, in most cases, the reverse is true; the larger depolarisation of the total scattering in liquids is due to the fact that the reduction of the intensity per molecule of depolarised orientation scattering is not so great as that of the polarised density scattering in passing from vapour to liquid.

FIG. 2

Spectrum of light scattered in (*a*) molten and (*b*) solid naphthalene
(Photograph by R. Norris)

Thus, the phenomena of light scattering give no support to the idea often expressed that liquids have a micro-crystalline structure. Neither is such a supposition true for the amorphous solid state. On the other hand, there is good reason for the belief that the liquid and the amorphous solid states stand in the closest relation to each other. The well-known fact that liquids may sometimes be super-cooled without crystallisation occurring and made to pass over into the glassy condition is a *prima facie* indication of that such a relationship exists.

The study of light scattering enables us to obtain a much deeper insight into the nature of the liquid and solid states of aggregation when, as was done by the present writer early in 1928, the mono-

chromatic radiations of the mercury arc lamp are employed and the aid of the spectroscope is enlisted for such studies. The changes of frequency then observed in the scattered radiations are of two different kinds in the case of liquids. Firstly, we have new lines appearing in the spectrum with fairly large and discrete frequency shifts; secondly, a continuous spectrum of radiations with altered frequency is also noticed. The latter effect is most conspicuous in the vicinity of the original lines of the mercury arc, taking the form of "wings" stretching out in the spectrum somewhat unsymmetrically on either side of these lines. To describe this phenomenon as a broadening of the spectral lines, as has been done by some writers, would be incorrect. Fig. 3 represents the group of four lines in the vicinity of 4358 A.U. in the light of the mercury arc scattered by benzene liquid and photographed with a Hilger 3-meter spectrograph. It is seen that the "wing" has its maximum intensity at the wavelength of the incident radiations; its presence, however, does not influence the spectral width of these radiations. As is evident from the figure, these radiations when not overexposed continue to be seen as lines of extreme sharpness in the spectra.

The origin of these "wings" has been the subject of much discussion. The experimental evidence which has accumulated, however, clearly supports the explanation of the same suggested in 1928 by the present writer and K. S. Krishnan. In the first place, the wings are fully depolarised to the extent of 6/7 in all the cases so far studied. Secondly, they appear in the same region of moderately small frequency shifts as that in which the rotation of the molecules in compressed gases and vapours and in liquid hydrogen records itself in light scattering. Thirdly, the "wings" are most intense in liquids containing ions or molecules which have a high degree of optical anisotropy, *e.g.*, fused inorganic nitrates and aromatic compounds. Fourthly, when the substance is in the crystalline state, the wing disappears, and is replaced by discrete lines exhibiting a wholly different distribu-

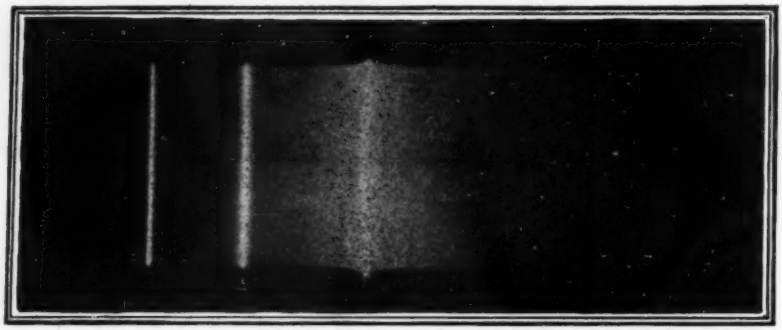

FIG. 3

Rotation " wings" in the spectrum of light scattered in benzene liquid
(Photograph by C. S. Venkateswaran)

tion of intensity (see Fig. 2). Fifthly, when the liquid is cooled down into the amorphous solid state, the wings become very weak and practically disappear. These observations clearly indicate that the "wings" are principally in the nature of "orientation" scattering and arise from the rotation of the molecules or molecular groups within the liquid. Here, again, the facts give no support whatever to the hypothesis of a micro-crystalline structure for liquids.

We now proceed to consider the character and origin of the scattered radiations which are recorded by the spectroscope in the same position as the radiations of the mercury arc. As stated above, the "wings" accompanying these lines represent depolarised orientation scattering. The question naturally arises, do the undisplaced lines consist only of polarised density scattering or do they include also any depolarised orientation scattering? This question is obviously of great importance and has been exhaustively investigated at Bangalore by Mr. B. D. Saxena and more recently also by Mrs. K. Sunanda Bai. These authors used a Littrow prismatic spectrograph by Hilger with a high resolving power to analyse the transversely scattered radiations and determine their state of polarisation. With the spectrograph slit opened very wide, the depolarisation of the total scattering by the liquid could be determined and came out practically the same as that observed without spectroscopic analysis. The slit was then made very fine to give the full resolving power of the instrument, and thus enable the depolarisation of the "undisplaced" scattering to be measured. It was found that in every case this was partially polarised and that the difference between the "wide slit" and "narrow slit" values of the depolarisation ratio depended greatly on the viscosity of the liquid and its temperature. The difference was found to be quite small for highly viscous liquids, *e.g.*, formic acid, benzophenone and glycerine, and much more marked in mobile liquids as also in viscous liquids at high temperatures. It was clear from the observations that the "undisplaced" scattering included a notable amount of depolarised orientation scattering, the proportion of this to the polarised scattering increasing with the viscosity of the liquid as well as with the optical anisotropy of the molecules contained in it.

In order to further elucidate the nature and origin of the "undisplaced" radiations observed in light scattering, C. S. Venkateswaran and more recently also K. Sunanda Bai have carried out elaborate studies, using a Fabry-Perot etalon for a spectral analysis of these radiations and a Lummer-Gehrcke plate to determine their state of polarisation. Investigations of this kind are not easy, and only by a careful choice of suitable interferometers and of the light sources and the most exacting care in experimental technique is it possible to obtain reliable results. It is not surprising, therefore, that these new investigations fail to confirm many of the conclusions stated by earlier workers in this field and in fact throw a completely new light on the subject.

A few remarks may appropriately be made here regarding the thermodynamic theory of light-scattering. Einstein considered the density fluctuations to be static and isothermal, while in the theory of

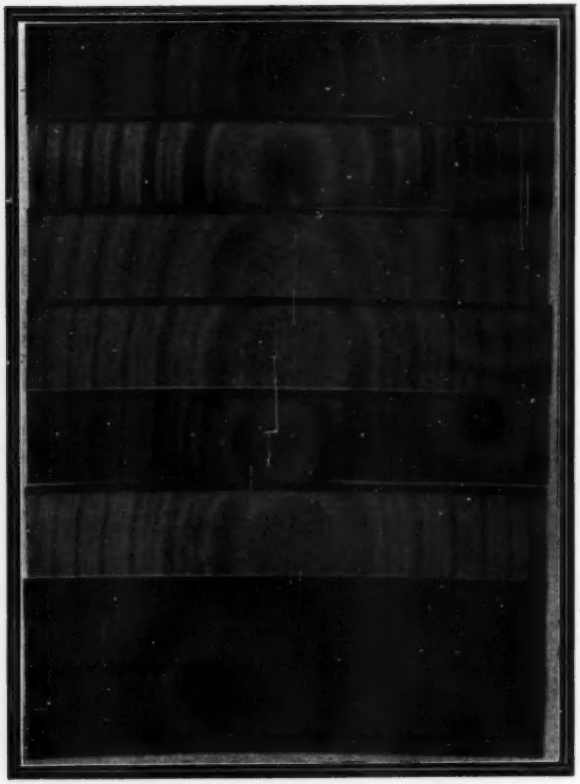

FIG. 4

Fabry-Perot patterns of light scattering in the alcohols (4078 A.U. Hg arc)

(*a*) methyl, (*b*) ethyl, (*c*) *n*-propyl, (*d*) *iso*-propyl, (*e*) *n*-butyl, (*f*) *iso*-butyl, (*g*) *iso*-amyl, (*h*) allyl alcohol

(Photographs by K. Sunanda Bai)

Brillouin they are considered as dynamic stratifications or sound-waves and therefore presumably adiabatic in character. The assumption that the fluctuations of refractive index arising from the density fluctuations are connected with them by the well-known Lorentz formula is neither necessary nor justifiable. The adiabatic piezo-optic coefficient of several common liquids has been recently determined at Bangalore by the present writer and K. Venkataraman. Using these coefficients and assuming the density fluctuations to be adiabatic in character, Sunanda Bai has recalculated the intensity of light scattering given by the thermodynamic theory and compared it with her own observations. When the presence of the depolarised orientation scattering is taken into account, the observed intensities are found to support the adiabatic hypothesis rather than the isothermal one.

It must not, however, be concluded from this approximate agreement of the observed and calculated total intensities that the thermodynamic theory of light-scattering is either correct or complete. The Fabry-Perot patterns of mobile liquids obtained by Venkateswaran exhibit a continuous band overlying the central or undisplaced component and the outer or Doppler-shifted components indicated by the Brillouin theory and suggest that these are imperfectlv resolved. In less mobile liquids, the components are more clearly separated. A central component is observed in all cases and is found to exhibit a clearly noticeable defect of polarisation, while the outer components appear to be polarised, though whether such polarisation is complete is open to doubt. The relative intensities of the central and outer components appear to

be controlled by two distinct factors, one being the viscosity of the liquid and the other the optical anisotropy of the molecules. The more viscous the liquid, the stronger the central component relatively to the outer ones. A greater optical anisotropy of the molecules appears to have a similar effect.

The outer or Brillouin components clearly owe their origin to the thermal agitation of the liquid. How does the central component arise? The most natural supposition is that it is due to the fundamental structure of the liquid which is akin to that of a glass or amorphous solid. This hypothesis is supported by the observation that its intensity relatively to the outer comopnents increases with the viscosity of the liquid, in other words, with the approach of the fluid to the amorphous solid state. Since the molecules would occupy fixed but random orientations in the amorphous solid, the presence of both polarised and depolarised components in the light diffused by it without any change of frequency is readily understood.

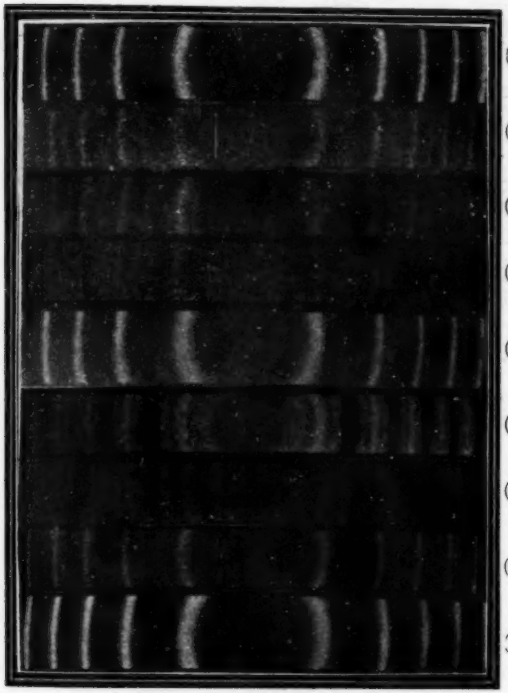

FIG. 5

Fabry-Perot patterns of highly viscous liquids

(*a*) Glycerine (0° C.), (*b*) Glycerine (26° C.), (*c*) Glycerine (42° C.), (*d*) Glycerine 110° C.), (*e*) Castor oil, (*f*) Cyclohexanol, (*g*) Glycol, (*h*) Phenol, (*i*) Styrol glass

(Photographs by C. S. Venkateswaran)

Further striking confirmation of these ideas is furnished by Venkateswaran's measurements of the hypersonic velocities in viscous liquids from the spectral displacements in the Fabry-Perot patterns. For ordinary or mobile liquids, the hypersonic velocity comes out practically the same as the ordinary ultrasonic velocity observed at much lower frequencies. But in the highly viscous liquids glycerine and castor oil, there is a marked difference between the two velocities and it is noticed that this difference falls off with rising temperature. A very satisfactory explanation of these facts is furnished by the consideration that for sufficiently high frequencies of vibration, a viscous liquid may to all intensities and purposes be regarded as an amorphous solid, and the hypersonic velocity should therefore depend both on its compressibility and the rigidity coefficient.

The experimental facts of light-scattering may thus be summarised in the statement that the basic structure of a liquid is the same as that of the corresponding amorphous solid, though disturbed and enlarged by thermal agitation. Precisely the same

conclusion is indicated by the study of the X-ray diffraction haloes (see Fig. 6) in liquid and glass. Indeed, the fundamental relationship between the phenomena of light-scattering and the X-ray effects was pointed out as long ago as 1923 in a paper by the present writer with K. R. Ramanathan. It is the same orderliness of molecular spacing which explains the diminished light-scattering which is also responsible for the diminished intensity of diffraction at small angles as compared with the vapour which is the most characteristic feature of the X-ray haloes of both liquids and amorphous solids.

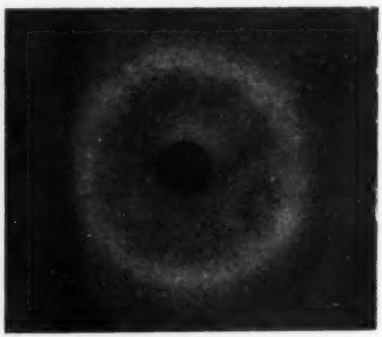

FIG. 6*a*

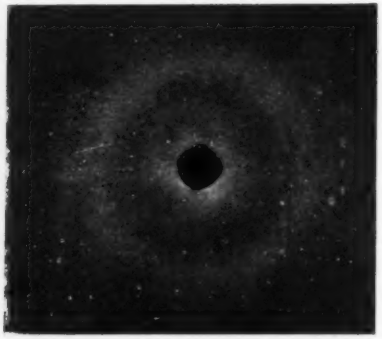

FIG. 6*b*

X-ray halo of glycerine, (*a*) glass and (*b*) liquid
(Photographs by T. M. K. Nedungadi)

C. V. RAMAN.

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

DR. SIR S. S. BHATNAGAR has made over a sum of approximately Rs. 13,000, which he has personally earned as royalties this year, to the Council of Scientific and Industrial Research for the purpose of financing research workers engaged on schemes of research in operation under the Board of Scientific and Industrial Research irrespective of whether the work is carried out under his direction at Delhi, or under the direction of any other recognised director of research, elsewhere in India. The help will be given to those workers who have earned no royalties, as their work had to be given in the interest of the war effort or development of industry. A part of this donation will be utilised for preparing an oil painting of the Hon'ble Dewan Bahadur Sir A. Ramaswamy Mudaliar, whose name is so intimately associated with the promotion of industrial research in this country. A sum of Rs. 1,000 will be donated to the Delhi University for encouraging scientific and industrial researches and a further sum of Rs. 2,880 has been earmarked for granting two scholarships of Rs. 60 each for two years in the Punjab University.

Messrs. Tata Sons have made a munificent grant of Rs. 8,30,000 to the Council of Scientific and Industrial Research for the construction and equipment of a National Chemical Laboratory. The Laboratory will be located at Poona and will be associated with the name of Tatas.

A donation of Rs. 1,00,000 to the Council of Scientific and Industrial Research has been made by Messrs. Indian Wire and Steel Products Ltd., for carrying out such work as may be agreed upon between the donors and the Council.

—(By courtesy of the Editor, *Journal of Scientific and Industrial Research.*)

THE CHEMISTRY OF THE ANTI-PERNICIOUS ANEMIA SUBSTANCES OF LIVER

BY

Y. SUBBA ROW

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THE efficacy of whole liver in the remission of pernicious anemia was first demonstrated in 1926, an observation which has since then been fully confirmed. In spite of several attempts by various investigators, the isolation of the active principle in a state of chemical purity and integrity has so far been unsuccessful but there has been a notable advance in the purification and potency of liver extracts. In view of the several divergent points of view regarding the chemical nature of the active material, it appears desirable to describe and review the work of closely allied groups of investigators together, before passing to that of another. This method of preparation, it is hoped, will, in preference to the strictly historical one, give a clearer perspective of the contributions of each worker in this field.

We shall not discuss the clinical aspects of pernicious anemia nor deal with the now widely accepted theory of the roles of the "extrinsic" and the "intrinsic" factors which, from a chemical view-point, have not so far been identified.

The claim of Klein and Wilkinson[47] that the active thermostabile principle of liver can be synthesized *in vitro* by incubating beef muscle and hog stomach extracts, could not be confirmed by Castle.[4]

We will not concern ourselves with the antianemic substance reportedly present in normal urine[88,76,15,77,53,43] nor attempt to decide whether the antianemic-principles found in the kidney,[59] lungs,[36] brain, salivary glands, saliva,[72] pancreas and other organs, are the same chemically as that associated with liver.

We will only refer to "a new therapy of pernicious anemia" with a spinach extract, a report, which is not supported by experiment.[64] No attempt is made to review the interesting observations of Massa and Zolezzi,[55,56,57] and of Mermod and Dock[60] on the use of congo red.

We shall deal only with the substance or substances in liver which exert a beneficial influence in cases of pernicious anemia.

Early Therapy and Assay

Before Minot and Murphy, there had been scattered suggestions in literature that pernicious anemia is a deficiency disease.[18,47] Whipple *et al.*, during the treatment of severe secondary anemia in dogs induced by repeated hemorrhage, observed the favourable influence exerted by the feeding of beef liver; they suggested that food factors be given serious consideration in the clinical management of pernicious anemia.[82,83] In 1926, Minot and Murphy reported the prompt and distinct improvement in a large number of pernicious anemia patients on a diet in which liver was an important constituent. Within two to eight days of such treatment there occurred an increase in the reticulocytes of the circulating blood, reaching a maximum on the third to tenth day and subsequently returning to the lower original level. With continued liver therapy, this reticulocyte response was followed by a rise in hemoglobin and total red cell count, with a return to an approximately normal blood picture in about two months. This blood response, chiefly the reticulocyte rise and return of red cells to normal number, has formed the basis of the clinical assay of potent preparations. Inasmuch as the chief difficulty in the work on the purification of active materials has been the relative scarcity of pernicious anemia patients, many worthy attempts have been made to develop an animal assay; the guinea pig, dog, cat, pigeon, swine, monkey and rabbit have all been tried without definite success in any. Creskoff and Fitz-Hugh have covered this subject admirably in their review on the standardization and assay of liver extract.[10] The clinical assay still is the only reliable way of following the fractionation procedures.

Fractionation of Liver by Cohn, Minot and Associates

In 1927, Cohn, Minot and their collaborators attacked the problem of the isolation of the active material from beef liver.[5,6,7,8,9] Table I summarizes their steps of fractionation. A major step in the purification procedure was the treatment of a concentrated

TABLE I

Inactive Fractions	Raw Minced Liver brought to pH 9·0	Active Fractions
Insoluble Residue (A)		Water Soluble Extractives brought to pH 5·0
Protein Precipitate (B) (acid-precipitable proteins)		Water Soluble Extractives heated to 70° C.
Heat Coagulable Proteins (C)		Water Soluble Extractives (D) extracted with ether
Ether Soluble Extractives (EE) [Removes 2 pct. of solids (D)]		Non-ether Soluble Extractives (E) extracted with strong alcohol
Alcohol Soluble Extractives (F) [Removes about 30 pct. of solids (D)]		Alcohol Precipitable Extractives (G) dialyzed
Dialyzed Extractives (H)		Dialysate (I) treated with silicic acid gel (pH 5)
Extractives Adsorbed by Silicic Gel (J)		Filtrate (K) extracted with *n*-butyl alcohol
Residues of *n*-butyl Alcohol Extraction (M)		Extractives (L) precipitated with basic lead acetate
Lead Precipitable Extractives (N)		Filtrate (O) precipitated with phosphotungstic acid
Filtrate from Phosphotungstate (Q)		Precipitate (P) phosphotungstates treated with 90 pct. acetone
Acetone-soluble Phosphotungstates (S)		Acetone-insoluble phosphotungstates (R)
		Starting with precipitate (P) regenerated and treated with 95 pct. alcohol
Insoluble Residue (peptones, proteoses, polypeptides)		Extract concentrated
		Intravenous Extract "I.E."
		"I.E." dissolved in 90 pct. alcohol, added equal volume of ether
Filtrate (contains tryptophane, tyrosine)		Precipitate. Treated with 1 volume H_2O: 9 vol. alcohol: 4 vol. ether
Precipitate (large number of substances giving diazo test)		Filtrate. Treated with 1 vol. H_2O: 12 vol. alcohol: 6 vol. ether
Filtrate (extracts phosphorus-containing substances)		Precipitate (Z). Treated with 11 vols. alc.: 6 vol. ether: 1 vol. H_2O.
		Precipitate (this is the fraction that has hitherto been precipitated by $HgSO_4$ in acid solution). Treated wtih picric acid.
		Precipitate (extremely active). (Effective in 140 mg. dose).

aqueous extract of liver (D) with an amount of absolute alcohol to result in a final alcohol concentration of 95 per cent.—the active precipitate is Cohn's Fraction G—which proved effective when fed daily in amounts of 9–14 grams (equivalent to about 200–400 grams of whole liver). A later modification was the treatment of the aqueous extract with alcohol to 70 per cent., the active principle remaining in solution with the precipitation of a good deal of inactive solids. They were able to eliminate proteins, fats and carbohydrates from the raw beef liver without a noteworthy loss in activity. The purer fractions contained less phosphorus and a greater percentage of nitrogen leading to the inference that "the active principle is either a nitrogenous base or a polypeptide".[6] On purer fractions the biuret test was negative and hydrolysis produced no increase in amino nitrogen. In highly purified solutions, heavy metals appeared to destroy activity. They believed that the active principle is free from proteins, carbohydrates, lipoids, tryptophane, tyrosine, arginine, cystine, creatinine, iron, sulfur and phosphorus.[8] Cohn's group concluded in 1930[8,9] that the active principle was a nitrogenous base, the nitrogen in which exists as in a secondary or tertiary amine. The low nitrogen content (10·8 per cent.) appeared to exclude purine or pyrimidine bases, but not ring compounds of the pyrrole or pyridine types. It seemed unlikely that it was a pyrrole because it gave no pine-splinter test characteristic of certain pyrroles. Their most active preparation (picric acid precipitate) proved active when given parenterally in a 140 mg. dose.

Early Work by West and Associates

West and Nichols in 1928[78] reported a product which showed a positive biuret test, a weakly positive Hopkins-Cole reaction and a positive test for arginine. West and Howe in 1930[79,80] prepared a product which proved active on intravenous injection of 680 mgm.; their best fraction appeared to be rich in a nitrogenous body with acid properties which on hydrolysis set 50 per cent. of its nitrogen free as amino-nitrogen. A crystalline quinine salt which at first seemed active clinically turned out later to be inactive.[81]

Later Work by Dakin, West and Associates

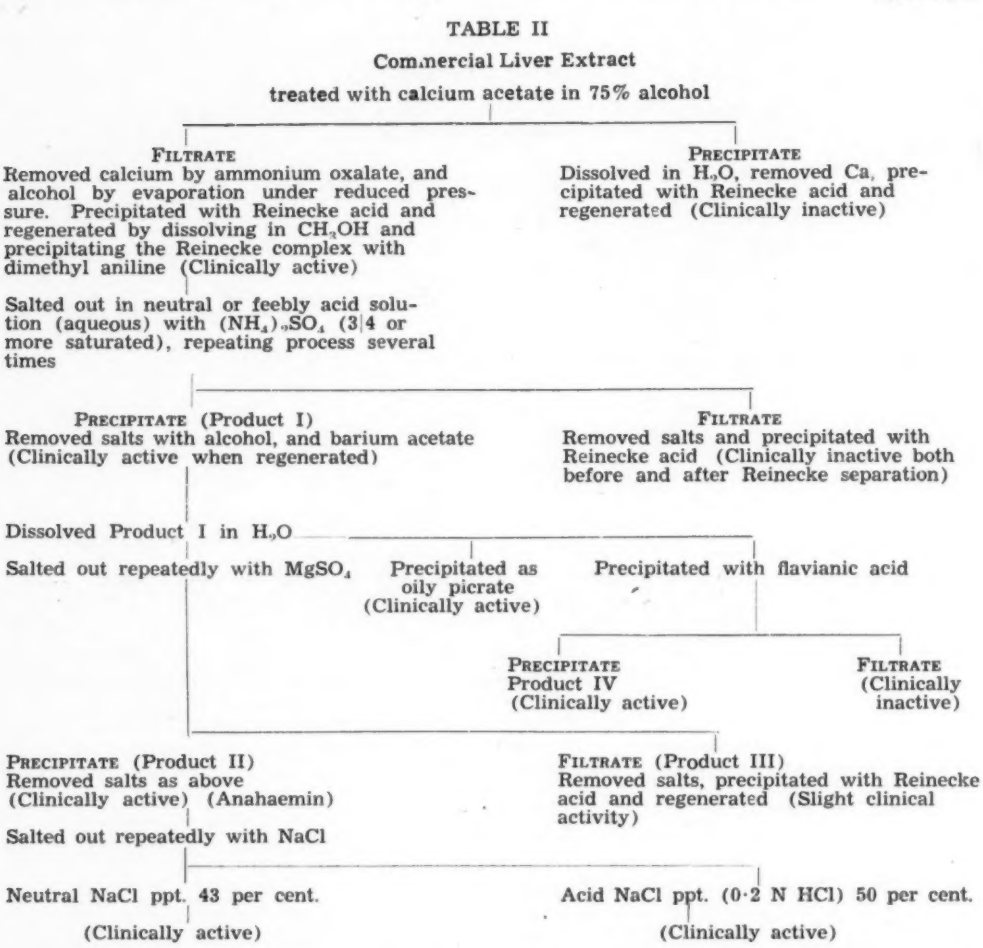
In 1935 Dakin and West[12] introduced the precipitation of the active material by Reinecke salt in acid solution, with subsequent removal from a weak alcoholic solution of the Reinecke acid as the sparingly soluble salt of a tertiary base (*e.g.*, dimethyl aniline) or by extraction with amyl alcohol. Table II summarizes the steps in their fractionation. 80 mg. of their Anahaemin gave a maximal clinical response. Hydrolysis of this active preparation yielded a group of amino acids. Complete absence of pyrimidine and purine bases, pentoses and desoxyglucose was reported. Later work by Dakin, Ungley and West in 1936[13] convinced them that the amino-hexose (glucosamine), which their previous preparation contained, was not an integral part of the active principle; they obtained further purification by introducing the precipitation of the active material by uranium (Table III). Their purest preparation at this time yielded on hydrolysis: arginine, glycine, leucine, aspartic acid, hydroxyproline and perhaps proline. There was also indication of a dicarboxylic acid easily soluble in water and giving a very soluble copper salt precipitable by alcohol—possibly hydroxyglutamic acid. From dialysis experiments through membranes of known pore diameter, they tentatively assigned a molecular weight between 2,000 and 5,000. The conclusion reached by Dakin, Ungley and West, as stated in 1936, was "that the hematopoietic substance in liver is, or is associated with, a peptide, possessing many but by no means all of the properties of an albumose". Recently Dakin and West[14] reported a few experiments on the precipitation of their material with albumose precipitants, including nucleic acid bile, taurocholic and other bile acids, and the barium carbonate reaction of Seigfried; the first three reagents yielded precipitates containing much active material.

Work of Laland, Klem, Strandell and Associates

Strandell and associates in 1935 and 1936[66,67] have reported the clinical assays of materials isolated by Laland and Klem.[52] Their procedure of fractionation—as summarized in Table IV employs phenol for the elution of the active material after adsorption on charcoal.

Dakin and West in 1935 found they could salt out the active substance by three-fourths to complete saturation with ammonium sulfate. Laland and Klem also found that

TABLE II



such treatment of their fractions yielded active material, although the antianemic principle was not precipitated quantitatively even by full saturation.

By a series of steps, the exact details of which have not yet been reported, Laland and Klem have obtained an active fraction "BBaBFu.s.E", 0·00035 grams of which correspond to 100 grams of liver. This material is a bright reddish-yellow, non-crystalline acidic substance, easily soluble in water, partly soluble in alcohol and insoluble in ether. They reported absorption bands in two regions of the ultraviolet range at 2500–2650Å and 3450–3500Å. The ninhydrin reaction was negative; the orcin test, positive. After hydrolysis, amino nitrogen as well as acidic and basic amino acids have been detected.

53 mg. of Heptomin II (corresponding to about 200 grams of liver) showed antianemic activity when injected intraglutially.[66] Their purest preparation "BBaBFu.s.E" when administered parenterally gave a satisfactory hematopoietic response in a dose of 0·7 mg. (corresponding to 200 grams of liver).[67] The Scandinavian workers regard their product as a biuret negative peptide.

Application of Laland and Klem Procedures to Dakin and West Material

Ungley in 1936 further purified an active Dakin and West fraction by the methods of

TABLE III

Liver Extract Power

1 kg. dissolved in warm H_2O (2·5 L); added $(NH_4)_2SO_4$ (1·4 kg.); in refrigerator overnight

ACTIVE	INACTIVE
PRECIPITATE	**FILTRATE**
Washed with saturated $(NH_4)_2SO_4$ soln. and dried; stirred in H_2O (500 c.c.); filtered	
FILTRATE	**RESIDUE**
Reprecipitated by saturation with $(NH_4)_2SO_4$	
PRECIPITATE	**FILTRATE**
Suspended in H_2O (200 c.c.); added alcohol gradually (500 c.c.); in refrigerator	
FILTRATE	**PRECIPITATE**
Concentrated under diminished pressure (less than 50° C.); added basic lead acetate (200 gm. in saturated aqueous soln.) and NH_4OH until solution was alkaline to litmus; filtered immediately	(of ammonium sulfate and alcohol-insoluble peptide)
FILTRATE	**PRECIPITATE**
Acidified to Congo red with H_2SO_4; filtered to remove $PbSO_4$; precipitated active material with Reinecke acid (about 25 gm.); regenerated the Reineckate; added uranium acetate (150 c.c. saturated solution)	
CURDY PRECIPITATE	**FILTRATE**
Washed well with cold H_2O; dissolved in H_2SO_4 (0·5 N); added $(NH_4)_3PO_4$ (2 gm.) and neutralized with NH_4OH to remove uranium as its insoluble phosphate; solution again subjected to precipitation with basic lead acetate (15 gm.), freshly prepared lead hydroxide (5 gm.), and NH_4OH added so long as a precipitate was formed.	
FILTRATE	**PRECIPITATE**
(Contained no glucosamine) Peptide recovered by acidifying with H_2SO_4; filtered off the $PbSO_4$; precipitated with Reinecke acid (3 gm.); regenerated the Reineckate; concentrated the decomposed Reineckate and precipitated with absolute alcohol	On decomposition with H_2SO_4, contained all of the glucosamine present in the product of the previous step.
SOLID PEPTIDE	
(4·02 gm.) (Glucosamine-free)	

TABLE IV

FRESH HASHED LIVER

Extracted with H_2O with addition of acetone to 50 pct. by volume (tissue H_2O inclusive)

ACTIVE | INACTIVE

EXTRACT I
strongly concentrated

CONCENTRATE (II)
filtered

FILTRATE (III)
freed from protein—different methods used: precipitation by metal sols or gentle heat coagulation with the addition of acid

PROTEIN-FREE FILTRATE (IV) (PERNAMI I)
free from albumin but not from salts. 3-4 gm. dry matter from original 100 gm. liver shaken with phenol—this extract, (IV) gives off the active substance quantitatively to phenol along with other substances. Add ether and H_2O to the phenol solution—the antianemic substance can be quantatively shaken into the water layer

FRACTION VI (Clinically very potent (HEPTOMIN—I—dark)

0·27 gm. dry matter from 100 gm. liver
Treat with active "coal" (charcoal)

COAL ADSORBATE
extracted by phenol and regenerated by shaking with ether and H_2O

FRACTION VIII (Fully active)
0·028 gm. dry matter from 100 gm. liver combined the phenol extraction with extraction with H_2O containing phenol

RESIDUE

FILTRATE

FRACTION B
0·001 gm. dry matter from 100 gm. liver additional adsorption on active charcoal and combined extraction with phenol water

FRACTION BB
0·001 gm. dry matter from 100 gm. liver evaporated to dryness, dissolved in glacial acetic acid and precipitated with excess ether

FRACTION BBa
(nearly colorless)
0·001 gm. dry substance from 100 gm. liver

FRACTION A
0·019 gm. dry substance from 100 gm. liver (inactive)

Laland and Klem.[75] Further purification was achieved by employing phenol and methyl alcohol for fractionation. 50 and 75 mg. of this purified preparation gave satisfactory results. By these methods Ungley purified Dakin and West's Anahaemin at least two and one half times.

In 1936 Wilkinson[86] applied Reinecke acid precipitation as employed by Dakin and West to the preparation of active liver extract[84, 85, 86, 47]; he was able to elicit maximal response with total doses of 18 to 36 mg. equivalent to 661 to 1332 grams of fresh liver.

WORK OF SUBBA ROW, JACOBSON AND ASSOCIATES—THE MULTIPLE FACTOR HYPOTHESIS

The investigators whose work we have so far considered have worked on the general hypothesis that the active portion of liver is a single chemical entity. The fact that

the reports of the chemical properties of their active preparations differed might lead to some doubt that a single substance is involved. The possibility exists that the activity of material effective in the treatment of pernicious anemia rests upon a certain type of compound or linkage common to more than one substance as is believed to be the case of some of the vitamins. Then again, one cannot neglect the consideration that successful therapy may depend upon the interaction of several factors.

In 1935 Fiske, Subba Row and Jacobson presented results which suggested that therapy in pernicious anemia could be achieved most successfully by a combination of two or more substances.[22,68] This led to the development of a multiple factor hypothesis. A report by Jacobson and Subba Row in 1937[41] indicated that there is (*a*) a primary, active hematopoietic factor in liver, and (*b*) at least three accessory factors, in themselves inactive, but whose presence materially augments the activity of the primary substance. They observed that continued purification of active liver extract, in the absence of significant losses and of destructive procedures, led to a partial or complete loss of therapeutic activity, but that combining these purified preparations of reduced potency with certain inactive fractions, resulted in the restoration of activity.

The four factors believed to be concerned are:

A. The primary factor of unknown chemical nature active in amounts of 0·2 to 0·4 mg. per day.

B. Three accessory factors: Inactive, singularly or in combination
   (1) Fraction A—*l*-tyrosine[68]
   (2) Fraction C—probably a complex purine[68]
   (3) Fraction F—probably a peptide.[70]

Fraction C (11 mg. from 100 grams of fresh liver) consisted of light yellow crystals showing positive xanthine, murexide and diazo tests and containing 33·1 per cent. nitrogen. The tentative conclusion was drawn that the substance is a complex purine resembling members of the pterine series of Wieland and Schöpf. Later work by Subba Row indicates that fraction C is composed, for the most part, of xanthine, accompanied by several other difficultly separable substances.

In 1936, Subba Row and associates[69] reported the preparation of an active product by the elution with 65 per cent. ethyl alcohol after adsorption on charcoal—a method which they had already used in the isolation of fraction C[68] and one which is quite similar to that developed by Kyer at about the same time.[51] A summary of the method of purification employed by Subba Row *et al.* is given in Table V.

Fraction I, a white microcrystalline material, had a negative biuret, Mellon's and Sagakuchi's tests; its absorption spectrum showed an inflection between 2480 and 2560 Å (note similarity to absorption spectrum of material of Laland and Klem). Fraction I proved effective clinically in total amounts of 4–8 mg. administered over a 10-day period.

In 1938, these investigators could report that by further purification the yield of total solids in the primary factor had been reduced to 1·2 mg. from 100 grams of liver, however with diminished potency.[71] There was evidence that the diminished potency was due to the absence of additional accessory factors. Two such materials were isolated from the mother fraction of the primary factor and identified as tryptophane and guanosine. All five accessory factors together, but without primary factor, were inert in a pernicious anemia case. Yet administration of the five accessory factors along with the primary factor (in dosage of 0·12–0·26 mg. per day) proved active; of eight cases four responded maximally.[71,42]

Further purification of the primary factor by readsorption on charcoal, elution and then precipitation by a mixture of alcohol and ether yielded an amorphous material which when tested on one patient showed good activity in dosage of 0·06 mg. per day. Chemical properties of the primary factor suggested that it is a complex pyridine derivative; in support of this view, synthetic nicotinic acid administered parenterally in dosage of 1 to 2 mg. per day to two patients along with three accessory factors (A, C and F) effected moderate hematopoietic response and clinical improvement.

Other Evidence for More Than One Factor

Eisler, Hammarsten and Theorell,[17] using cataphoresis, obtained evidence of two active principles in liver preparations—one of which leads to reticulocytosis and the other,

TABLE Va

150 c.c. *Commercial Liver Extract* (equivalent to Cohn's G)
(3 c.c. from 100 gm. fresh liver)
Dissolved in 1 liter H_2O
Brought to pH 8 with NaOH, acidified to pH 6 with HCl
Added 50 grams norite and stirred 1 hour
Filtered

Charcoal + Adsorbate
Washed repeatedly with H_2O till washings colorless.
Suspended in 1 liter 65 per cent. ethyl alcohol, brought to the boiling point, stirred 5 minutes and filtered hot
Elution repeated

Combined Eluates
concentrated under diminished pressure at 40° C. to 150 c.c.

Fraction B
concentrated further under reduced pressure

White Granular Material crystallized

Fraction C

Filtrate—Fraction D

TABLE Vb—I

Fraction D
(150 c.c. from 5 kg. liver)
(10-12 mg. total N per 100 gm. liver)
Acidified to pH 2 with HCl
Added 16 gm. fuller's earth
Stirred mechanically for 30 min. at room temp.
The ppt. was filtered and washed once with 50 c.c. H_2O

Filtrate + Washings
Added 10 volumes 95% ethyl alc. and 10 vols. ether
Mixture left in cold room 24-36 hours
Filtered

Precipitate (Fraction H)
(20 mg. from 100 mg. of liver—12-13% N)
Dissolved in 50 c.c. H_2O
Brought to pH 3 with 10 N H_2SO_4
Caused a crystalline ppt. to settle, mainly of $CaSO_4$

Filtrate
Added to 40 c.c. H_2O containing 1 gm. Reinecke salt at 40°
In cold room for 24 hours—brought down a crystalline precipitate
Filtered

Precipitate
Washed once with 50 c.c. ice cold H_2O
Suspended in 300 c.c. 0·03 N H_2SO_4 at 30-35°
The Reinecke acid was removed by repeated extraction with a 500 c.c. mixture of equal vols. of amyl alcohol and ether

Concentrated in vacuo to a volume of 25 c.c.

Concentrate
Added 10 vols. acetone and 10 vols. ether
In cold room 48 hours

Precipitate (Fraction I)
(Yield of 100 mg.—*i.e.*, 2 mg. from 100 gm. fresh liver)

TABLE Vb—II

Fraction D (1 liter)
Added 7 liters 95% ethyl alcohol
At room temp. 5 hours
Filtered

Filtrate
Added 3 liters ethyl alc. and 10 liters ether
In cold room 48 hours

Precipitate (Fraction E)
Dissolved in 500 c.c. H_2O
Added 200 c.c. 5% solution of rhodanilic acid in methyl alcohol
In cold room 48 hours
Filtered

Crystalline Precipitate
Freed of rhodanilic acid by pyridine with subsequent removal of pyridine by ether

Regenerated Rhodanilate Solution
Precipitated by Reinecke salt
Reineckate regenerated (as in other method)

Fraction I
(Yield about the same as in other method)

when administered with the first, to erythropoiesis as well. Hofer in 1934[36] separated a "reticulocyte response" factor from a general "curative" factor.

On the Importance of Tryptophane and Histidine

From the peptide character of the active material of Dakin and West, and of fraction F of Subba Row and associates as well as their evidence for the importance of tyrosine and tryptophane, and from the observations of Laland and Klem that amino acids are among the products of hydrolysis, it would appear that amino acids are of importance in the problem of pernicious anemia therapy. The use of tryptophane in the treatment of anemia (experimental) is first credited to Hirazawa.[34] In the early 1930's, Fontes and Thivolle presented evidence in favour of the hematopoietic action of both tryptophane and histidine.[24–32] They regarded the former as the precursor of the tetrapyrrole group of hæmatin and the latter as the amino acid essential for the formation of globin in the hemoglobin molecule. They first showed the hematogenic action of tryptophane and histidine by their observation that subcutaneous injection into normal rabbits and dogs resulted in "hyperhemoglobinemia" and "hypererythrocytosis"; this action seemed to depend upon the presence of indol and imidazol nuclei and not to be a function of amino acids in general. Alcock in 1933[1] could not produce anemia in experimental animals by tryptophane deficiency alone and was thus led to doubt that the pyrrole of hæmatin is derived from tryptophane. In 1936 Hamada[33] confirmed the results of Fontes and Thivolle by producing an anemia in rats on a tryptophane-poor diet. Levi has reported that the injection of tryptophane in rabbits with experimentally produced anemia caused a restoration of the red cells and hæmoglobin to nearly normal values.[54]

Fontes and Thivolle reported results from the administration of histidine and tryptophane to pernicious anemia patients. They believed that the digestive disturbances in pernicious anemia may interfere with protein breakdown. Their treatment of six cases[29] with parenteral administration of 200–400 mg. histidine and 100–200 mg. tryptophane for a month was followed by a rapid remission of long duration in one case, a slow remission in another, a rapid remission followed by relapse in the third, and complete inactivity in the other three cases—not an impressive record in a disease which may run a course of remissions and relapses as pernicious anemia does.

Fontes and Thivolle have consistently maintained that the activity of raw liver and of the various liver extracts depends entirely on their contact of tryptophane and histidine in the free state. However, Cohn and associates obtained active preparations from which all tryptophane had been removed. Tryptophane and histidine were not found among the amino acids set free on hydrolysis of the material of Dakin and West. Aleksandrowicz and Gabryelski[2] found no tryptophane in a commercial preparation of proved activity (Pernaemon).

Negative results were obtained by Cuthbertson, Fleming and Stevenson,[11] who gave daily injections of 100 mg. tryptophane and 200 mg. histidine to two pernicious anemia patients. Dominici and Penati[16] were also unable to confirm the favourable results of the French investigators. Tochowicz[73] agreed with Fontes and Thivolle that some of the trouble in pernicious anemia lies in faulty protein metabolism; he concluded that although tryptophane may play a role in pernicious anemia, histidine is of no importance in either the pathogenesis or the treatment of the disease.

Here again there are conflicting reports. Tentatively, we may conclude that tryptophane may play a role in the treatment of pernicious anemia, yet by no means a major one. The evidence for the importance of histidine in the "antianemic factor" is even less convincing.

Work of Mazza and Penati

Mazza and Penati[58] have isolated active materials, containing, they believe, a nucleotime, a polypeptide and a pterine. Their steps of fractionation are summarized in Table VI.

Their substance C which they believe is of pterine nature showed maximal absorption at 2535 Å and 2490Å.

Substance D contained 3 per cent. ash (traces of iron and copper); its microanalysis showed 50·1% C, 7·6% H and 12·4% N. It also contained phosphorus (about 3 %) and sulphur (trace). Ninhydrin and Mellon's tests were weakly positive; there were strongly positive tests for histidine and pentose; and a negative test

TABLE VI

Minced pig liver
Pressed at 500 atmospheres pressure
Liver juice
Treated with 96% ethyl alcohol to give a 70% alcohol content
Dilute H_2SO_4 to pH 5
Boiled for ½ hour

Precipitate (proteins)

Filtrate
Concentrated in vacuo to a syrup
Extracted with trichlorethylene

Extract (lipoids)

Syrupy residue
Taken up in 80% alcohol

Precipitate

Filtrate
Neutralized
Treated with saturated soln. of $CaCl_2$

Precipitate

Filtrate
Calcium removed by ammonium oxalate

Precipitate

Filtrate
Solvent removed in vacuo
Treated with 5 vols. 98% alcohol
Precipitate dried in vacuo

Substance A
(500 mg. from 500 gm. fresh liver)
(active in total dose of 3 grams)
Put in aqueous solution
Treated with soln. of basic lead acetate and $Ba(OH)_2$

Filtrate
Pb and Ba removed by H_2SO_4
Filtrate to pH 5
Charcoal adsorption

Charcoal and Adsorbate
Elution with warm (70°) 50% ethyl alcohol at pH 5 (acetic acid)

Eluate
Alcohol removed in vacuo
Charcoal adsorption and elution repeated
Resulting eluate evaporated to dryness

Substance B
(100 mg. from 500 grams liver)
(active in total dose of 0·5-1·0 grams)
(sky blue fluorescence)

4% *Solution Substance B in* H_2O
Precipitated with H_2SO_4 and phosphotungstic acid

Precipitate
Decomposed by $Ba(OH)_2$, the Ba being removed by H_2SO_4 solution concentrated to small volume
Treated with 10 vols. absolute alc. and 10 vols. acetone

Precipitate
Dissolved in H_2O
Saturated with $(NH_4)_2SO_4$

Precipitate
Dissolved in 75% alcohol and reprecipitated with absolute alcohol and acetone
Precipitate dried

Substance D
(5 mg. from 100 grams liver)

4% *Solution Substance B in* H_2O
to pH with HCl
Adsorbed on fuller's earth
Eluted with 5% pyridine at 70°

Pyridine Extract
Evaporated to dryness
Residue dissolved in 1 N NaOH
Acidified with HCl

Filtrate
Adsorbed on fuller's earth and eluted with 5% pyridine
Eluate concentrated in vacuo to small volume
Added NH_4OH, NH_4Cl and $AgNO_3$

Precipitate
Put in solution with 1N HCl
Solution adsorbed with fuller's earth which is then eluted with 10% pyridine
Pyridine eluate evaporated in vacuo to dryness

Substance C
(1 mg. from 5 kg. liver)

for tryptophane. It showed maximal absorption at 2600 Å and 2650 Å. Of the total nitrogen, 5·8% was amino nitrogen; after acid hydrolysis for 1½ hours amino nitrogen accounted for 68·2% of the total nitrogen. They believe that fraction D consists of a combination of an adenine nucleotide and a polypeptide containing proline, oxyproline, histidine, arginine, hydroxyglutamic acid and a monoamino-monocarboxylic amino acid.

Unfortunately, Mazza and Penati have as yet not reported adequate clinical tests for their material. In the single case which they do report for their purest preparations (C & D), substance D given alone (375 mg. over a period of 8 days) showed no activity. Then in the same case both D (total additional dose of 325 mg.) and C (total dose of 13 mg.) given over a two-month period showed only moderate activity, raising the red count from 2·0 million to 3·2 million. From this one case considering the relatively large amount of material used, their substances are most likely not very pure and thus the chemical properties they have described may be those of contaminating compounds rather than of the antianemic factor.

Miscellaneous Contributions

Buchanan in 1929[3] reported that the active principle of liver extracts has apparently every chemical resemblance and physiological action of oxidized glutathione. Although Fleming[23] later declared that glutathione, mostly in oxidized form, enters into the composition of the antianemic factor, Koser in 1936[50] denied the importance of glutathione in this respect.

In 1933 Felix and Fruhwein[21] fractionated active liver extract, following the activity by determining the reticulocyte response after injection in anemia (not necessarily pernicious anemia) patients or normal persons, and by methemoglobin formation *in vitro*. After repeated purification with mercuric sulfate, their preparation gave a negative test for tryptophane, and a negative biuret test. It is unfortunate that their preparations were not adequately assayed on pernicious anemia patients.

In 1935, Erdös[19] reported his work on the preparation of active material. He made an acid extract of finely hashed liver, the protein then being removed by iron. Inasmuch as his material gave a positive biuret and showed an increase in amino-nitrogen by about 300 per cent. after acid hydrolysis, he believed it to be a peptide. He isolated a water insoluble silver salt from the analysis of which he suggested the formula $C_{650}H_{720}O_{36}N_{30}S_2P_2Ag_3$ with a molecular weight of about 10,000. Unfortunately his method of assay was inadequate; he tested activity on the anemia produced in dogs and rabbits by the administration of phenylhydrazine.[20]

We have thus far discussed the results obtained by investigators who employed the orthodox chemical procedure of isolation and identification. In 1937, Jacobs[37,38,39] reported conclusions regarding the chemical character of the active liver material which he reached by employing less orthodox means. From a study of the properties exhibited by potent liver extracts, he tried to deduce the chemical nature of the antianemic principle. In his first paper[37] he suggested further investigation of the possible role of glucosamine and the common amino acids (especially leucine and tyrosine). By mixing raw potato scrapings source of tyrosinase) and tyrosine he believed he could form the 5, 6-quinone of dihydroindole-2-carboxylic acid, the so-called "red substance" of Raper[65]; feeding such a mixture to a pernicious anemia patient resulted in inconclusive results.[39] Later Jacobs concluded that the "red substance" of Raper was not concerned in the activity of liver extracts. In his most recent paper[40] he proposes to investigate choline for its antianemic effect inasmuch as he was able to isolate this compound from a commercial liver extract. However, we doubt that this substance will show any activity at all; we are of the opinion that the activity of the commercial liver extract was lost for the most part during his repeated decolorizations with animal charcoal.

Aleksandrowicz and Gabryelski[2] have proposed that heparin is one of the important therapeutic factors in liver preparations. Heparin administered intramuscularly to three pernicious anemia patients effected a moderate erythrocyte response in one. On the whole their results, both clinical and chemical, do not present convincing evidence for their thesis.

Jones, Phillips, Larsell and Nokes[44] reported that nuclear extractives from various

organ extractives—supposedly consisting chiefly of nucleoproteins and the sodium salts of nucleic acids—yielded satisfactory results in pernicious as well as other anemias when administered orally.

Karrer, Frei and Fritzsche in 1937[45] reported that liver preparations possessing full antianemic potency in a singly administered dose of 10–20 mg. contain amounts of phosphorus, pentose and adenine consistent with the presence of an adenin-nucleotide. However, a purer preparation active in doses of 8 to 10 mg.[46] contained no phosphorus or pentose. The ninhydrin test was positive, before and after hydrolysis, but the biuret was negative or at best only weakly positive. No polypeptides of the usual type were present. After hydrolysis, 2-amino acids were present only in small quantities, if at all. Koller[49] reported on the clinical use of Karrer's material.

In a short note in 1939, Tschesche and Wolf described the properties of an active material that resembled the preparation of Karrer *et al.* in that it showed a negative or only slightly positive biuret test, but a positive ninhydrine both before and after hydrolysis.[74] Their material, a white powder active in a dose of 40 mg., also had a negative Molisch and a negative Millon test; it was free of flavine, purine, pterine, reducing sugar and phosphoric acid esters. It analyzed: C, 50%; H, 7%; N, 14·5%; S, 0·6%. They have not as yet reported their clinical results in any detail.

Comparison of Activities of Preparations

In the foregoing sections, the methods of preparation and, in some cases, the properties of various antianemic preparations have been described. There is as yet no agreement among the different investigators regarding what is the active material, or what are its properties. It may be of some interest, however, to compare the relative activities of some of the better preparations. How should such a comparison be made? The phenomenon of reticulocyte response is of little value in determining the comparative efficiency of extracts. It may or may not give an indication of the presence of potency, and it does not appear to indicate the degree of potency. Murphy suggests[63] that perhaps the most critical and important means of comparison is the determination of the amount of antianemic material that is necessary to maintain the erythrocyte count of a pernicious anemia patient at a normal level. Inasmuch as there are few reports available concerning the long-time maintenance requirements of the extracts in which we are interested we cannot make our comparisons on this ideal basis.

In the absence of a more satisfactory basis of comparison, a few of the preparations may be compared in terms of the erythrocyte response elicited by definite quantities of different products (Chart 1, from *J.A.M.A.*, 1941, 116, 367).

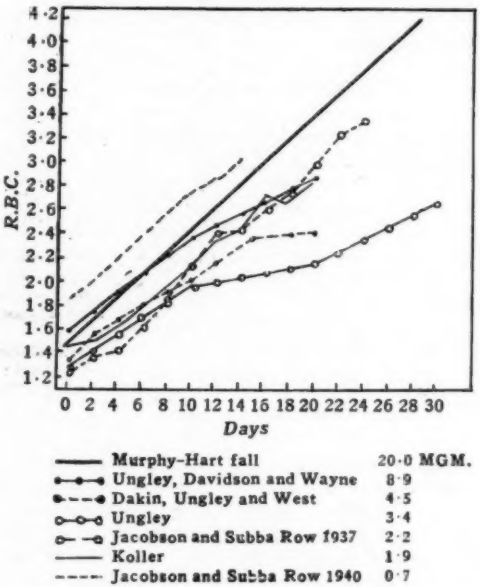


Chart 1.—Average erythrocyte regeneration curves following the administration of various purified liver extracts. The calculated average daily amount of material administered is recorded in the legend. The sources of these data are contained in the text. (The daily doses recorded for the curves of Jacobson and Subba Row refer only to the amount of primary factor administered; in addition, three accessory factors were administered in the 1937 curve, in a daily amount of 3·4 mg. and five accessory factors were administered in the 1940 curve in a daily amount of 6·2 mg.)

The data have been presented in the hope that further advances in the understanding of the chemical nature of the substances concerned will enable us to explain the discrepancies in the magnitudes of response achieved by different investigators. It is,

TABLE VII

Comparison of reported properties of certain liver extracts containing primary anti-pernicious anemia factors

	Cohn *et al.*	Dakin *et al.*	Laland *et al.*	Subba Row *et al.*	Karrer *et al.*
PHYSICAL PROPERTIES					
Color	....	....	Reddish Yellow	Colorless	....
Solubility	Sol in H_2O " " Acetic acid " " Phenol " " Formamide " " Glycerol " " 70% Alcohol Slightly sol. in 95% Alcohol Insol. in Abs. Alc. " " Ether " " Acetone	Insol. in Absolute Alcohol	Insol. in ether Sol. in H_2O Partly soluble in alcohol		....
Insoluble Salts with	Phosphotungstic Acid Tannic Acid Sulfuric Acid Picric Acid Gold Chloride Platinic Chloride Silver Nitrate	Pptd. by High Conc. of Trichloracetic acid but not by Low. Not pptd. by Rufianic Acid. Not pptd. by Ferrocyanic Acid. Not pptd. by Metaphosphoric Acid.	Phosphotungstic Acid Heavy Metals		....
Precipitation by $(NH_4)_2SO_4$	.. .	Precipitated	Partially pptd.	....	....
Dialysis	Dialyzable	Dialyzable	Dialyzable	Dialyzable	....
Specific Rotation	....	$(a)^{16}D$–112° to –133°	....		
Absorption of Light	....	....	250–265 mu ; 345–350 mu	248–256 mu	....
CHEMICAL CONSTITUTION					
Composition in per cent.					
C	....	46·8 –48·1	53·64	41·56	45·68
H	....	6·6 – 6·8	6·85	6·74	6·75
N	10·8	15·2 –16·8	13·33	13·13	14·63
S	None		0·74	1·2	Present
Ash			2·05	(SO_4 –10·2)	
Molecular Weight	....	2000–5000	....	....	
Presence of Pentose	None	. ..	Present	Present	None
Biuret Reaction	Negative	Negative	Negative	Negative	Negative
Amino Hydrogen					
Before Hydrolysis	None	0·5		·5	
After Hydrolysis		10–10·4	Not Reported	75	Not Reported
Amino Acids Isolated	Not Reported	Arginine, Glycine, Leucine, Aspartic Acid, Hydroxyproline, Proline	Not Reported	Not Reported	Not Reported

unfortunately, apparently not possible at the present time to reconcile the various claims and facts regarding the material or materials which are present or capable of extraction from liver, and which are therapeutically active in pernicious anemia. Table VII summarizes the chemical properties of some of these preparations which we have discussed. Although it is not yet possible to present the nature of the active material with chemical exactness, it is, nevertheless, proper to note with some satisfaction that since whole liver therapy was instituted, the amount of material needed by the patient per day had decreased from 400 grams to less than 10 mg. So much

progress makes it reasonable to expect the isolation of the active material in the near future.

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CANCER RESEARCH IN INDIA

BY

V. R. KHANOLKAR

(From the Department of Laboratories, Tata Memorial Hospital, Bombay)

THERE is probably no other disease or group of diseases regarding which there are so many misconceptions in the minds of the medical and general public as cancer. It may therefore be useful to state some general ideas regarding the nature of cancer, which have evolved as a result of clinical observation and experimental investigation during the last 30 years before dealing with cancer research in India.

Cancer is the expression of a universal cell property and includes a great group of diseases which differ widely from one another in origin, clinical course and morphological appearances. This conception that cancer is not a single disease, but a generic term covering a broad field of biological science is now accepted by all who have studied this problem carefully. If this conception is correct it follows that one should not look for similar course in the evolution of different types of cancer, nor is it profitable to look for one single cause or one particular type of treatment for the various types of malignant disease. One might therefore neglect as unreasonable the constantly recurring announcements in the general press of the so-called discoveries of the cause of cancer as well as its cure and which always receive warm but short-lived reception from men of large means but small understanding.

If the view that unrestrained and malignant proliferation is a property inherent in all cells is acceptable then it follows that every cell should be capable of such proliferation under certain specific conditions. A good deal of clinical and laboratory evidence has now accumulated which supports such a view and it has been found that so far as human subjects are concerned no cell type is exempt from a neoplastic growth. Careful observation has shown that all races of men who have been studied are liable to cancerous growth in one form or another. It has further been observed that malignant tumours occur in most members of the vertebrate species and that even insects are not exempt from it. The same variety of predisposing, contributory and exciting factors and the same general biological properties are revealed in lower animals as in man. This property of unrestrained growth is evidently more marked in certain types of cells, *e.g.*, embryonal cells. The cells in atrophic or vestigeal organs are also prone to an unrestricted autonomous growth. It is known that this property is influenced by heredity, and that the suppressed growth of cells in adult life is liable to be released from restraints by certain external influences or agents, such as injuries, inflammations and radiations. Modern studies conducted by the use of cancer producing chemicals have shown that conditions which obtain in healing wounds favour the appearance at the site of injury of progressively growing tumours. In some experimental animals it has been found that the occurrence of breast cancer usually depends upon the working of three separate factors operating at the same time. (1) A hereditary factor, or susceptibility. (2) A factor transmitted in the milk of animals with a tendency to cancer production. (3) A hormone related to pregnancy.

It is a noticeable fact that interest in research on cancer has been almost completely neglected in this country both by members of the medical profession and by workers in basic sciences like Biology, Physics and Chemistry. And yet one cannot conceive of any other field of study in human disease which affords such wide scope for investigators in these sciences. This lack of interest may be attributable to several reasons.

Firstly, an impression has been fostered in the minds of the public, by people who have not spent much time or thought on this matter that once a person is declared to be suffering from cancer, his case is hopeless and nothing much could be done to get him over his ailment. I remember reading a paper many years ago before medical men assembled from all parts of

this country, on the incidence of different types of cancer in patients who presented themselves in one of the hospitals in Bombay. The audience was impressed but baffled and could not understand that any one should waste his time on collecting information of this type. Their feelings were expressed by one of the members who rose to ask a question at the end of the paper. He wanted to know the use of all that had been said unless he could be told as well how to cure cancer. One did not know then and one does not know now of a cure for all cancer, but a great deal is known about the treatment of different types of cancer and a cure by rigid standards can be demonstrated in a large number of cases.

The second reason is that in most of the countries which have a poorly developed health organisation, and a health consciousness in the masses of people is almost lacking, the infectious diseases like cholera, plague, typhoid, small-pox, parasitic diseases like malaria, kala-azar, amœbiasis, and nutritional deficiency diseases overshadow the picture of diseases in the country to such an extent that cancer does not occupy an important place in the minds of medical profession. This does not mean that cancer is less frequent here than in other countries. Hardly twenty years ago it was a current belief among many people that cancer was a disease of civilisation meaning European civilisation and it was therefore argued that uncivilised or non-European countries should have hardly any cancer. There was no justification for this belief except for the fact that in economically rich countries the successful control of infectious and parasitic diseases increases the normal expectation of life and many more people live long enough to suffer from diseases peculiar to middle and old age. In economically poor countries like India a large bulk of people do not live long enough to show the manifestations of cancer which are mainly noticeable in the fourth decade of life and even later.

The third reason is probably an inadequate training of members of medical profession regarding the basic facts of cancer. A significant change has however taken place in the attitude of the medical profession towards malignant diseases, inasmuch as there is a growing recognition of the deficiencies of the medical knowledge about cancer and its treatment. It has been realised that effective study and treatment of cancer may no longer be considered the occasional task of the general practitioner or surgeon, but constitutes a highly complex medical speciality. It may safely be said that cancer is now owned to be one of the most important and most progressive departments of medicine, as it is also the most fundamental problem of biology.

Cancer research, like most medical research, follows three main directions. At first a disease is noticed and clinical observers study its manifestations, its points of resemblance and the characteristic differences which distinguish it from other similar conditions. Thus by careful continued observation a lot of useful information is collected which supplies the basis for diagnosis, and treatment of a clearly delimited disease entity. So far as cancer is concerned medical men have observed its manifestations in human beings for many centuries and attempts have been made to limit its ravages by salves, ointments and internal medications with very doubtful efficacy. It is only during the last forty years that important information has begun to accumulate as a result of a careful record of all the signs, symptoms and complications, along with a careful microscopical study of tumours, and a long follow-up of cases extending over five and ten-year periods. Such work is laborious and is unattended by any spectacular results in a short time. It can only be undertaken in properly organised institutions. In many institutions in Europe and America it has yielded a rich harvest of knowledge which has revolutionised our ideas regarding the nature and treatment of malignant disease. To cite only one example many types of cancer which were considered hopeless at the commencement of this century are now successfully treated at the New York Memorial Hospital and large numbers of people are saved from the slow but relentless ravages of a dread disease. In this country cancer has been treated more or less efficiently in most of the big medical institutions; however the system of records and follow-up is so woefully defective in the majority of places that no useful contribution has been made to the sum of our knowledge, in spite of the skill of our surgeons and the scientific attainments of

our physicists and radiologists. Isolated observations have been recorded in some medical journals which relate mainly to the unusual types of malignant disease. A newer consciousness is dawning among the younger medical men and it is hoped that a spirit of conscientious team work which is gradually developing will replace a statement of vague impressions by an accurate description of personal observations. So far as the non-medical public is concerned it may be stated that all careful observation has shown that cancer is not incurable, and that in early stages presents a much more hopeful outlook than some other diseases which hold no dread to the public such as diabetes, heart disease and nephritis.

It may not be out of place to say a few words about the so-called cancer cures which are extensively advertised by the press and the credulous public from time to time. Malignant disease is difficult to detect in its early stages without long and adequate training and a systematic study. On the other hand many cases which are diagnosed as cancer are probably inflammatory in nature and improve with ordinary care and treatment. It is possible to relieve the acute discomfort and reduce the swelling attending cancer by several methods without eradicating the disease. In such cases though the disease is advertised as cured, it recurs after a lapse of months or years and may lead to a fatal termination by its appearance in some other organ in the body. The danger to the public lies in the fact that the patients in their attempt to follow some plausible treatment deprive themselves of the opportunity of being effectively treated at a stage when a radical cure could be expected by modern methods.

The second approach to the problem of cancer is by a statistical investigation of the cancer morbidity and mortality in any particular country over a stated period of time. Until as recently as the commencement of this century knowledge was so slight and contemporary statistics so inadequate, that dogmatic statements were made and believed by people who were guided by feelings and impressions. One of the first points, therefore, which had to be cleared, was to determine whether any race of man is really immune from neoplastic disease, as had been confidently asserted. The immediate importance of the inquiry lay largely in the possibility that by this means information could be obtained pointing to significant effects on cancer incidence of environment, diet, social conditions or racial character. Such investigations have produced valuable results in countries where the vital statistics are accurately maintained and where the economic conditions permit every citizen to obtain medical relief either from individual medical practitioners or from private or public medical institutions. Such information has allowed a study of particular types of malignant diseases being associated with certain occupations or certain social habits. It has further been found that certain types of cancer are more prevalent in certain regions and much less common in neighbouring areas where the habits or economic condition of inhabitants are not very different. These differences in the frequency and types of malignant disease have stimulated investigation into their causes and have in many cases been fruitful in results. The frequency of cancer of the lip in luminous dial painters, the cancer of the skin in chimney sweepers and the cancer of lung in miners in Czecho-Slovakia are a few instances of this type. In our country statistical investigations are handicapped by several factors. The certification of a large number of deaths by non-medical men, the lack of adequate medical facilities except in larger towns, the easy credulity of many educated and most ignorant people are factors which make the work of public health administrators well-nigh impossible. However, in spite of these drawbacks several interesting observations have been forthcoming which deserve further study. The frequency of Kangri cancer of the skin of the abdomen in Kashmir described by Neve,[1] the cancer of the cheek in betelnut chewers on the south-west coast of India described by Orr,[2] and the cancer of external genitalia in the Hindu males are conditions which deserve careful study. A valuable preliminary statistical study was undertaken by Vishwanath and his colleagues[3] from Lahore on the basis of hospital records in the big medical institutions in this country. This is the only instance of an investigation on cancer in this country which was assisted

by a grant from the Indian Research Fund Association.

The third method of approach to the cancer problem is by laboratory experimental method. This method was delayed because of the great difficulty experienced by early workers in transmitting malignant disease to experimental animals or in producing cancer by external applications. A new era of cancer research was initiated by the work of Yamagiwa[4] and his pupils after production of cancer of the skin in rabbits by applications of tar in 1914. Since that time an enormous amount of work has been done on experimental transmission of cancer in animals, the histological changes in the skin by application of carcinogenic substances, and as a result of exposure to X-rays and gamma radiations, the chemistry and synthesis of carcinogens, the production of mammary cancer in mice by administration of hormones. The Rockefeller Institute has been working on the question of viruses in the production of certain types of cancer in animals, and the work of Rous and Murphy has thrown much light on this difficult problem. The question of heredity in cancer has been studied by Slyle in Chicago, Little at Bar Harbour and Lynch in New York. The experimental work has assumed such proportions that it has become impossible for one person to keep acquainted with all its phases. There is still a tendency to ignore the fact that experimental cancer research like most other branches of scientific endeavour has made remarkable progress especially during recent years and it seems that great possibilities for further work have been opened up which will elucidate the nature of the cancerous process in the near future. In this country experimental research on cancer has not been touched upon until quite recently and it is expected that with the facilities available at institutions like the Tata Memorial Hospital, the contribution of India in this research will not be negligible. Already some interesting work on the relation of certain enzymes to cancer susceptibility has been carried out and the study of extrinsic factors in production of cancer is engaging the attention of workers. After the termination of the present unsettled conditions this work will gain impetus and many young men will be coming forward to tackle fundamental problems on cancer research from different angles of approach. It is not unreasonable to hope that research foundations and public endowments will give adequate assistance to young men who have the necessary training to undertake investigations at recognised institutions on cancer problem. It need not be emphasised that without such assistance original work on cancer will be impossible in this country and we shall continue to wait as passive spectators in the memorable march of scientific progress.

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INDIAN INSTITUTE OF SCIENCE

THE Government of Mysore have made a capital grant of Rs. 1 lakh and a recurring annual grant of Rs. 15,000, for the institution of æronautical and automobile engineering sections at the Institute.

The President of the *Hindustan Aircraft Construction Co., Ltd.*, has offered a scholarship of $3,000 a year, for four years, to a student of the Institute, tenable at an American institution, for higher studies in Aeronautical Engineering.

—(By courtesy of the Editor, *Journal of Scientific and Industrial Research.*)

LETTERS TO THE EDITOR

A CONSIDERATION OF THE SUCCESSIONAL THEORY OF TEETH

When I first disputed (1938) the oft-repeated statement that the hinder teeth of sharks were there for replacement I was referred to William Andre's report of an injured jaw of the "tiger" shark. An illustration of this appeared in an article Dr. E. W. Gudger kindly sent me but this I regarded as convincing proof that the abnormal teeth there described were due, not to any progression of the gum after the injury, but to disturbance of the dental germs at the injured site.

A photographic illustration of erosion in a marine rock remarkably resembled what sometimes is seen in sharks and rays. Although the former was rightly attributed to depression which allowed accumulation of rain and subsequent erosion of the rock, without question of revolving of the surface, attempts are made to explain what occurs in the jaw of sharks and rays to constant forward movement of the covering of the jaw-cartilage for replacement of teeth which are supposed to be constantly shed.

Examination of sharks at various stages of growth failed to reveal evidence of shedding except for an occasional exposed tooth, whilst minute teeth at the foremost position of the teeth of some sharks rendered very remote the possibility of their being replaced by the stouter hinder ones. Nor did it seem likely that the hindermost poorly developed teeth would ever move forward to replace those stout but seldom used teeth which are usually covered by gum unless this is torn away.

As such replacement by forward progression is quite impossible in some sharks, such as the Devil Ray, it seemed a mistake to conceive some means of replacement other than that of vertical succession and, for want of experimental proof, investigations were made to discover whether any other animals than sharks could replace their teeth in any way but by vertical succession.

Embryonic and adult skates and rays revealed evidence to show that forward progression of the tooth-bearing area was very unlikely to occur, the gum being closely attached to the underlying cartilage and the flattening of the front teeth being constant throughout life, and unrelated to wear and tear.

No species of fish showed revolving of the tooth-bearing area, vertical succession being

demonstrated by X-ray examination and by dissection of the jaw. Thus stout rounded teeth of one row would never move into the position of a sharp tooth of another row when the latter was lost.

Lizards similarly showed vertical succession of their teeth where such replacement was possible and minute teeth lying loose in the gum appeared to correspond to the hinder teeth of fishes and the little loose teeth of Pythons, being rather for reinforcement than for replacement.

Replacement of the solid teeth of snakes did not seem to occur. Careful observation failed to reveal new tooth formation at the base of a tooth and there seemed to be a constant number of teeth in each row of a fully developed example of each species.

Possible replacement of the hollow-teeth of back-fanged snakes appeared to be impossible, for there seemed to be no provision made for revolving forward of undeveloped fangs and the loss of a fang behind a row of solid teeth must be infrequent.

The only position in which it seemed possible to apply the successional theory was in that of the grooved teeth of the front-fanged snakes, where serial development also lent support to the belief that a lost fang might be replaced.

However, occasional ancylosis of an extra fang is not necessarily connected with replacement and it was impossible to conceive how reserve fangs could move into such a position as to emit poison from the only duct of a poison-gland, once a function fang was lost.

These studies indicate that replacement of teeth under natural conditions occurs only by vertical succession, and that the successional theory, involving a constant forward movement of the tooth-bearing area, is founded on assumptions for which there is no justification.

F. Gordon Cawston.

West Street,
Durban, S. Africa,
July 7, 1942.

ARC LINES OF COPPER IN FLAME SPECTRA

Eder and Valenta who were the first to investigate such spectra, have reported the presence of the following lines amongst others of copper: line λ 4651 A. in the Bunsen flame and lines λ 4275 A. and λ 4355 A. in the oxycoal-gas flame. All these lines involve negative energy levels in the copper atom. The lines λ 4651 A. and λ 4275 A. have the energy level $(-95{\cdot}2\ \text{cm.}^{-1})\ 3d^9 4s(3D)\ 5s\,^4D_{7/2}$, as their initial level. The line λ 4355 A. similarly involves the negative energy level $(-2951\ \text{cm.}^{-1})$ $3d^9 4s(^3D)\ 5s^2D_{5/2}$. The existence of these lines, if real, would point to the availability of an amount of energy of the order of about 7·7 e.v. (7·68 i.p. of cu + 95 cm.^{-1}) in the Bunsen flame and 8·04 e.v. in the oxycoal-gas flame. The possibility of obtaining copper atoms in a state higher than its first ionisation potential in the Bunsen flame was thought to be rather remarkable. In our previous note we also have noted the presence of the line λ 4651 A. on our plates. Since that note was published, however, spectra have been taken with an instrument of higher dispersion and resolution. These show very clearly that the line does not belong to the copper atom at all but is one of the strong structure lines or possibly a condensation of many such lines near the head of the (0-1) CuH band at λ 4650 A., whose head is at λ 4648·5 A. and whose origin is at λ 4661·8 A. Fig. 1, which is a contact print of

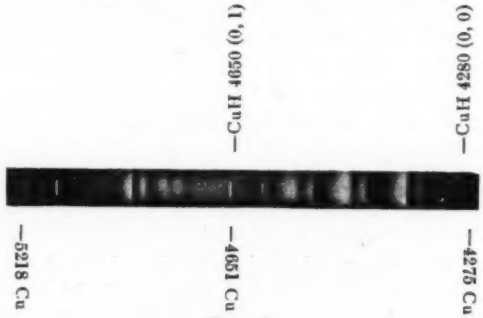

Fig. 1

the copper-chloride flame spectrum (inscribed in which is the copper arc spectrum) taken on

the three prism glass spectrograph shows this very clearly. The wave-length of the copper line itself being λ 4651·1 A., the mistakes in the earlier literature are understandable. We have not repeated the experiments with the oxycoal-gas flame but it seems likely that the lines at λ 4275 A. and λ 4355 A., given by Eder and Valenta are also probably structure lines of the CuH band (0,0) at · 4280 A. and of the bands (1,1) or (2,2) respectively. There does not seem, therefore, to be any experimental evidence for the existence of negative energy levels of copper atom in flames fed with copper salts.

NAND LAL SINGH.

B|1, University Quarters,
Benares Hindu University,
Benares,
July 7, 1942.

[1] *Proc. Ind. Sc. Congress*, 1941, p. 31.
[2] *Atlas Typischer Spektren*, Eder and Valenta, Wien, 1911.
[3] *Atomic Energy States*, Bacher and Goudsmit, 1932.
[4] *Report on Molecular Spectra*, etc., Jevons, 1932.

ACCUMULATION OF PYRUVIC ACID IN RICE MOTH LARVÆ (*CORCYRA CEPHALONICA* STAINT) FED ON A VITAMIN B_1 DEFICIENT DIET

WHEN experimental animals like the rat or the pigeon are kept on a vitamin B_1 deficient diet, there occurs in the blood a marked accumulation of carbonyl compounds, chiefly pyruvic acid.[1,2] This characteristic change has also been observed in the blood, urine and cerebro-spinal fluid of persons suffering from beriberi.[3] In all these cases, administration of a suitable amount of vitamin B_1 brings about a rapid fall in the pyruvic acid content of the blood to the normal level.

In view of these observations, it was of interest to discover whether the larvæ of the common rice moth (*Corcyra cephalonica* Staint), for whose normal growth vitamin B_1 has been found indispensable,[4] show similar biochemical reactions. The larvæ, after being grown on whole wheat for 15 days, were transferred to a diet deficient in vitamin B_1. They were kept in an incubator at a temperature of 30° C. and a humidity of 75 per cent. They were weighed every week. It was found that the larvæ continued to grow for the first 15 days after which a falling off occurred in the growth rate. After 35 days, the pyruvic acid content of these insects was determined by Lu's method.[5] For comparison the pyruvic acid content of larvæ fed on whole wheat was determined. The effect on pyruvic acid content of transferring larvæ fed for 35 days on a vitamin B_1 deficient diet to a diet containing 5 microgrammes of vitamin B_1 per gramme, was also investigated.

The results are given in the table. For comparison, the blood pyruvic acid values in normal and B_1 deficient rats, pigeons and human beings are included.

	Pyruvic acid	Author
1. Larvæ on whole wheat	18·5–20·5 mg./100 g. dry wt.	
2. ,, B_1 deficient diet for 35 days	164·3 ,, ,,	
3. Larvæ from (2) transferred to a diet containing 5/B_1 per g. for 66 hrs.	88·25 ,, ,,	
4. Pigeon, normal	0·84 mg./100 c.c. blood	Thomson and Johnson
,, deficient	5·85 ,, ,,	Thomson and Johnson
5. Rat, normal	0·96 ,, ,,	Li and Kato
,, deficient	5·62 ,, ,,	Li and Kato
6. Human, normal	0·56 ,, ,,	Lu
,, deficient	2·35 ,, ,,	Lu

It will be seen that the larvæ, like man and the laboratory animals mentioned, accumulate pyruvic acid when fed on a vitamin B_1 deficient diet. The amount of pyruvic acid present was reduced by nearly 50 per cent. in 66 hours on the inclusion of vitamin B_1 in the diet. The amount of pyruvic acid in the larvæ, both in the normal and deficient state, is much larger than that present in the blood of either man, rat, or pigeon. This, together with the ease with which the larvæ can be handled and the rapid reproducibility of results, suggests the possibility of devising a quick and sensitive method for the estimation of minute quantities of vitamin B_1, such as might be present in blood and other biological materials. The method might be of value in assessing partial degrees of avitaminosis in man. Work along these lines is now in progress.

P. S. SARMA.
KAMALA BHAGVAT.

Nutrition Research Laboratories,
Indian Research Fund Association,
Coonoor,
August 4, 1942.

[1] Thomson and Johnson, *Biochem. Jour.*, 1935, **29**, 694.
[2] Li and Kato, *Jour. Lab. Clin. Med.*, 1940, **26**, 1314.
[3] Platt and Lu, *Quart. Jour. Med.*, 1936, **29**, 355.
[4] Swamy and Sreenivasaya, *Curr. Sci.*, 1940, **9**, 493.
[5] Lu, *Biochem. Jour.*, 1939, **33**, 249.

STUDIES IN INSECT NUTRITION: BIOLOGICAL ASSAY OF THIAMIN WITH *CORCYRA CEPHALONICA* STAINT AS THE EXPERIMENTAL ANIMAL

THE rice moth larvæ were fed *en masse* in batches of 25 on two diets—thiamin and thiamin-free—yeast being employed as the source of the vitamin. Thiamin-free yeast was obtained by autoclaving brewery yeast at 30 lbs. for 3 hours. Batches of 10 larvæ picked out at random from the culture dishes, were weighed after known intervals.

The results (Table I) reveal that autoclaving destroys a thermolabile growth factor, manifestly essential to the insect. This factor was suspected to be closely allied to, if not identical with, thiamin.

TABLE I

Diet	Weights of ten insects in mgm. after known intervals in days		
	15	25	35
Thiamin-free	1·16	1·21	3·40
Thiamin	3·74	29·50	166·00

With a view to elucidate this point, feeding experiments were conducted with thiamin-free diet (autoclaved yeast) to which known amounts of synthetic thiamin were added. Results are given in Table II.

TABLE II

Diet	Weights of ten insects in mgm. after known intervals in days						
	15	20	30	40	45	63	70
B_1 (active yeast)	2·89	7·0	51·67	205·5	236·5	..	..
B_1-free (autoclaved yeast)	1·9	2·0	2·15	..	..	..	..
Do. + 1·44 I.U. per gm. of diet ..	1·16	..	2·75	7·0	..	43·33	82·5
Do. + 2·88 I.U. per gm. of diet ..	1·45	1·7	4·5	14·4	16·9	83·33	104·2 (one pupated)
Do. + 5·76 I.U. per gm. of diet ..	2·4	3·5	8·85	28·0	33·5	139·2 (2 pupated)	All pupated

I.U. = International Unit.

It will be observed that from the data (Table II) that when the diet is supplemented with graded doses of thiamin, the insects grow and it is interesting to note that the increases of growth, are strikingly proportional to the amounts of thiamin added. This at once points to the possibility of employing these insects for an assay of thiamin in extracts, physiological fluids and foods.

It will also be seen (Table II) that the diet, to which the maximum amount of thiamin is added, does not promote the growth of the insect to the same extent as is achieved by the active yeast diet. This may be due to two causes:—(*a*) either the quantity of thiamin is not sufficient or (*b*) there is some factor other than thiamin, which is either destroyed or rendered unavailable to the insect during the process of autoclaving.

With a view to examine the first possibility, experiments on the supplementation of the vitamin to a thiamin-free diet, in graded amounts covering an extended range, were carried out; the results are given in Table III.

TABLE III

Thiamin per gm. of diet I.U.	0	5	10	15	20	40	Active yeast
Wt. of insects after 23 days in mgm.	High mortality	3·35	6·60	10·0	9·5	9·0	40·5

It will be seen from Table III that fifteen international units of thiamin per gram of diet, saturates the preparation; higher amounts of the vitamin do not induce further growth. But, when the diet is prepared with an equivalent quantity of active yeast, a fourfold increase in growth is obtained. This increase could not be attained by an exclusive addition of thiamin. As indicated, we are led to postulate the existence of another thermo-labile factor, an elucidation of the nature of which, is under active investigation.

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July 25, 1942.

COMPARATIVE STUDIES ON THE NUTRITIVE VALUE OF FISH AND PRAWNS MUSCLE

IN Bombay coastal waters, along with different varieties of fish (vertebrates) prawns (invertebrates) also are caught and utilised by the public in abundance. The fish are seasonal in their availability while the prawns are found throughout the year. As the prawns contain a high percentage of protein and form a staple food for the poor class in Bombay and along Konkan coast, it was thought desirable to study the comparative nutritive value of the prawn and fish. Four varieties of fish and four of prawns were undertaken to study their nutritive values. In addition to the determinations of the biological values of the proteins of prawns and fish some of the other constituents, *viz.*, fat, calcium, phosphorus and iron, have been estimated. Biological value was determined according to the method adopted by Mitchell,[1] and Chick *et al.*[2] The amount of fat was determined by extraction of the dried material with ether. The ash was estimated by Stolte's method described by Peters and Vanslyke.[3] Phosphorus was estimated by Brigg's[4] modification of Bell and Doisy method, and calcium by the volumetric permanganate method described by McCrudden,[5] and iron according to Kennedy.[6]

In Table I*a* and I*b* are given the analyses of the fresh fish and prawns and in Table II*a* and II*b* their biological values and digestibility coefficients.

TABLE Ia

Analysis of the fresh fish

Name	Scientific Name	Edible Portion	Contents per 100 grammes of the edible portion							
			Moisture	Protein	Fat	Ash	Calcium	Phosphorus	Iron	Insoluble Inorg. matter
Surmai	Cybium	87·2%	63·0	19·86	1·37	—	92·54	161·75	2·031	——
Ghol	Sciœnea	91·7%	69·7	18·39	0·898	2·37	88·57	153·2	2·059	0·035
Mushi	Scoliodon	94·95%	76·46	14·86	2·860	1·026	58·64	168·86	2·06	0·028
Ravas	Polynemus	92·60%	70·86	20·60	0·56	2·18	96·08	162·6	2·36	0·068

TABLE Ib

Analysis of the fresh prawns

Tendli	Metapeneus	100%	72·60	19·60	3·08	1·86	82·0	157·8	1·31	0·086
Sode I	Parapeneus	100%	73·89	21·41	2·68	1·26	92·0	176·0	2·30	0·060
Sode II	Parapeneus	100%	19·41	2·08	2·08	1·86	72·86	166·9	3·60	0·080
Golim	Acetes	100%	19·6	2·86	2·86	1·86	106·0	128·0	2·10	0·09

TABLE IIa

Biological value and digestibility coefficient of fresh fish

Name	Scientific Name	Level of Intake	Biological Value	Digestibility Coefficient
Surmai	Cybium	5%	75·56	84·96
		10%	67·97	81·18
		15%	59·37	76·14
Ghol	Sciœnea	5%	81·45	83·03
		10%	71·30	83·35
		15%	58·83	76·23
Mushi	Scoliodon	5%	72·88	82·18
		10%	62·14	84·19
		15%	53·26	70·75
Ravas	Polynemus	5%	79·5	85·87
		10%	67·96	85·16
		15%	52·20	67·52

TABLE IIb

Biological value and digestibility coefficient of fresh prawns

Name	Scientific Name	Level of Intake	Biological Value	Digestibility Coefficient
Terdli	Metapeneus	5%	71·80	86·39
		10%	65·67	85·81
		15%	59·62	73·19
Sode I	Parapeneus	5%	75·96	84·23
		10%	66·51	85·47
		15%	58·77	72·57
Sode II	Parapeneus	5%	78·19	85·72
		10%	74·85	87·09
		15%	60·84	73·22
Golim	Acetes	5%	75·64	83·63
		10%	60·74	86·03
		15%	54·46	71·87

The results show that both fish and the prawn muscle constitute cheap scurces of animal proteins and essential minerals such as phosphorus, calcium and iron. Further, the

proteins are found to possess high biological value and digestibility coefficient.

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S. C. DEVADATTA.

Wilson College,
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June 20, 1942.

[1] Mitchell, *J. Biol. Chem.*, 1924, **58**, 905.
[2] Chick *et al.*, *Biochem. J.*, 1935, **29**, 1702, 1712.
[3] Peters and Vanslyke, *Quantitative Clinical Chemistry*, 1932, **2**, 70.
[4] Brigg, *J. Biol. Chem.*, 1922, **53**, 13.
[5] McCrudden, *Ibid.*, 1909, **7**, 83 and 1911, **10**, 187.
[6] Kennedy, *Ibid.*, 1927, **74**, 385.

THE EFFECT OF AN ILL-DRAINED SUBSOIL ON THE GROWTH OF COTTON AND GROUNDNUT

IN the cotton tract of the Central Provinces and Berar cotton is grown over nearly 3 million acres of land varying in the nature of soil and subsoil. One of the common types observed is that of soils apparently fertile having an ill-drained subsoil, locally known as *chopan*, at varying depths from 6 inches downwards. The physical and chemical nature of this subsoil has not been studied fully and the cause of its impermeability not yet ascertained. The mechanical analysis shows its clay content to be varying from 45 to 48 per cent. and in general its physical composition is very similar to the surface soil, locally known as *morand*. Some of the physical characteristics of these soils are given below:

Soils	Apparent specific gravity	Capillary rise of water in inches				Percolation of water in inches		
		1 hour	1 day	1 wk	2 wks	5 min.	10 min.	15 min.
Morand	1·14	2·0	3·7	15·0	20·0	2·0	5·0	6·0
Chopan	1·23	1·0	2·0	6·7	8·5	0·5	0·7	1·0

The *chopan* layer forms a sort of hard pan which is more or less impermeable to water and roots of plants. It causes water-logging and also affects the plant growth, yield and root development.

A pot culture experiment was arranged to study the effect of *chopan* subsoil at 6", 9" and 12" depths on growth and yield of cotton and groundnut. Eight pots under each of the three treatments were filled with *morand* as surface soil and *chopan* as subsoil at depths of 6", 9" and 12" respectively (referred to as treatments 1, 2 and 3 respectively). Eight pots were filled completely with *morand* to serve as control (treatment 4). One set of 32 pots filled in the above manner was sown early in July with Verum 434 cotton and another set with groundnut Ak 12·24 variety. The plants were watered every alternate day and mulching and weeding were done once a fortnight. Fortnightly observations were taken on general vigour and height. Finally plants in one pot of each treatment were washed with a fine jet of water to study the root development. The following observations are of interest:

(1) The vigour of plants was satisfactory and almost the same in all pots upto the end of August when some differences were noticed. The plants in control pots were green and healthy while those in pots having *chopan* at 6", 9" and 12" depths showed slight yellowing.

(2) Even though the pots were irrigated on alternate days plants with treatments 1 and 2 showed early cessation of growth and tendency towards early maturity. The heights of the plants were in general less than plants under treatments 3 and 4 (control) and reached their maximum about 1 to 2 weeks earlier.

(3) The yield of cotton and groundnut ranged in the order of the depth at which the *chopan* subsoil was put in as shown in the following table. The yields in grams are the total of eight pots under each treatment.

The difference between any two treatments is significant and the controls have given the highest yield. This shows that the presence

Crop	Treatment I *Chopan* at 6"	II *Chopan* at 9"	III *Chopan* at 12"	IV All *morand*	Mean	S.E.
Cotton	47	52	79	81	64·8	0·96
Groundnut	474	511	561	685	557·8	3·12

of *chopan* layer nearer to the surface soil affects the yield adversely.

(4) The roots were found to grow easily up to the subsoil and then instead of penetrating the *chopan* layer curled upwards and formed a mesh (Figs. 1 and 2).

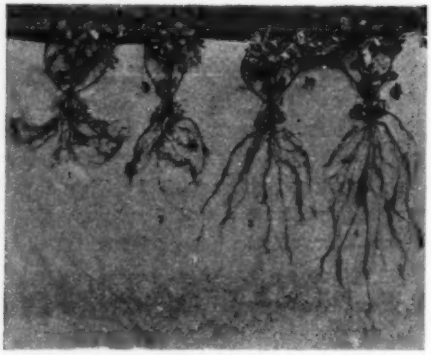

FIG. 1
Groundnut roots

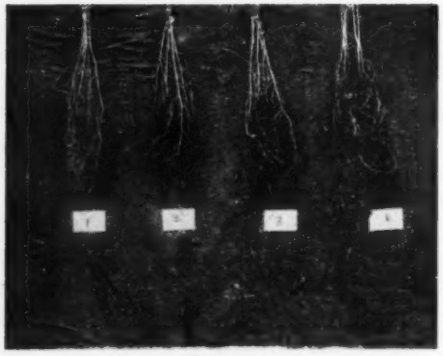

FIG. 2
Cotton roots

It is thus clear that the general performance of plants is dominated by the depth at which the *chopan* layer occurs. Further work on the problem is in progress.

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MAGNESIA AS AN ADSORBENT FOR ASCORBIC ACID

AN adsorption technique has been developed for the preparation of ascorbic acid from plant extracts and successfully applied to the case of the Indian gooseberry, *Phyllanthus emblica*.

A survey of promising adsorbents including metallic oxides and hydroxides revealed that both fibrous alumina and light magnesia serve as good adsorbents, the latter being by far preferable. MgO has been reported to form a compound with ascorbic acid.[1]

Phyllanthus emblica, the Indian gooseberry, contains 2·0-2·5 per cent. ascorbic acid calculated on the basis of air-dried, seed-free material. The vitamin content decreases on storage. The alcoholic extract contains 3·8 per cent. total solids of which 7 per cent. is accounted for by ascorbic acid, the rest comprising mostly of tannins and resinous matter. Magnesia preferentially adsorbs tannins, and by passing the alcoholic extract through a column of magnesia, an extract free from tannins and rich in ascorbic acid is obtainable.

Owing to its basic character, magnesia oxidises ascorbic acid, partially and irreversibly. The maximum recovery of the acid in our preliminary experiments was as low as 57 per cent. This difficulty was overcome by (1) employing a low temperature (0° C.) for adsorption, and (2) using magnesia calcined at a high temperature. High temperature (1000° C.) calcination, not only suppresses the basic character of magnesia but also enhances its adsorption capacity. The relation between temperature of calcination and adsorption efficiency, awaits detailed investigation. Calcined magnesia is known to occlude oxygen and for the success-

ful application of this adsorbent, it is necessary to cool it out of contact with air, or preparing a paste of the material with alcohol before bringing it into contact with ascorbic acid and subjecting it to vacuum to remove oxygen. By taking these precautions, the loss of ascorbic acid during the process of adsorption can be reduced to about 5 per cent.

The ascorbic acid can be quantitatively eluted by bubbling either H_2S or CO_2 through the suspension of the adsorbate in water. The ratio of ascorbic acid to the impurities in the eluted extract is 1 : 2 and the concentration of the impurities can be considerably reduced by repeating the adsorption.

The adsorbent can be recovered and used repeatedly after calcination. The method is being employed for the preparation of ascorbic acid on a large scale.

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P. R. Sivaramakrishnan.
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Bangalore,
August 19, 1942.

[1] Yamato and Takeshi Hara, *Bull. Agr. Chem. Soc. Japan*, 1940, **16**, 1038.

DISTRIBUTION AND FEEDING OF THE POST-LARVAL FISHES OF THE TRIVANDRUM COAST

From November to middle of March, post-larval stages of various species varying in length from 5 to 35 mm., appear along with larval stages of other species, while towards the end of March, the latter gradually vanish from the catches until in April post-larval fish alone are caught. The maximum abundance of these, however, is recorded in February-March. Russell[1] carrying out similar investigations in the Plymouth area observed that the maximum abundance of post-larvæ occurred during the period between the second fortnight in April and the first fortnight in June.

Post-larvæ of *Cybium commersonii*, *Engraulis commersonianus*, *E. tri*, *Equula blochii*, *Lactarius delicatulus*, *Pristipoma stridens*, *Scomber microlepidotus*, *Sillago sihama*, *Sciæna albida* (?), *Sphyræna jello* and *Therapon jarbua*[2] are usually caught along this coast. *Clupeatricauda*, *Stromateus niger*, *S. sinensis*, *Polynemus hepatadactylus* and *Upeneoides vittatus* are comparatively rare. However, each month has its own predominant species as may be seen from the following table:—

January—*Engraulis commersonianus*.
February—*Equula blochii*.
March—*Cybium commersonii* and *Scomber microlepidotus*.
April—*Sillago sihama*.
May—*Sphyræna jello*.

On the other hand, *Lactarious delicatulus* is uniformly found during November to May.

Analyses of the stomach contents of post-larval fishes mainly reveal two facts: (1) The feeding habits of the post-larvæ more or less foreshadow the feeding habits of the adults of the same species. (2) The young fish exhibit selective feeding to a remarkable degree. The plankton of this coast during November to March consist mostly of crustacean larvæ, copepods, Leucifers, Mysids, medusoids, lemellibranch larvæ and Pteropods. From March onwards there is a gradual increase in the diatoms.[4] Copepods, Mysids and Leucifers form the main items in the food of the post-larvæ of *Scomber microlepidotus* and *Engraulis spp.* Similarly in predacious forms like *Cybium commersonii* and *Sphyræna jello*, fish larvæ constitute the bulk of the stomach contents, even though small-sized crustaceans and other organisms abound in the plankton.

Adult specimens of *Lactarius* are known to be carnivorous, living on zoo-plankton organisms. Stomach contents of their post-larvæ (up to 15 mm.) commonly reveal a greenish mass, probably digested diatoms and occasionally complete specimens of *Coscinodiscus* and *Thalassiothrix*. Here we have a probable instance of alternative feeding. Such differences have been observed with European Herring.[3]

The presence of larval stages in October and the continued appearance of these up to March indicate that the spawning season of most of the marine fishes commences from September and extends to February or even March. *Equula blochii is probably* an early spawner and *Sphyræna jello* seems to be a late spawner.

Lactarius delicatulus seems to spawn intermittently for, larvæ and post-larvæ of this, greatly varying in length, are simultaneously met with all through the season.

Thanks are due to Dr. C. C. John, Professor of Marine Biology and Fisheries for his general guidance and helpful suggestions.

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July 7, 1942.

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[1] Russell, F. S., *Journ. Mar. Biol. Assoc.*, N. S., 1935, **20,** 150.

[2] Day's nomenclature is followed.

[3] Hardy, A. C., *Min. Agri. Fish. Fish. Invest.*, Ser. II, 1924, **7,** 3, 1–53; Lebour, M. V., *Journ. Mar. Biol. Assoc.*, N. S., 1924, **13,** 330 and Ogilvie, H. S., *Fisheries Scotland Sci. Invest.*, 1927, **1,** 1–10.

[4] Data kindly supplied by Mr. M. A. S. Menon of this Laboratory to whom I am greatly indebted.

REVERSE MUTATION IN *OPUNTIA DECUMANA*

THE prickly peer to which species of *Opuntia* belong has one species free from spine, called the spineless cactus. In this species when the joints (phylloclades) are young, tender fleshy spines are present, but these soon drop off, as the joint enlarges to form a spineless surface. The exact origin of the spineless cactus is obscure. Luther Burbank supposes that during the phylogeny of the cactus the original spineless type has acquired the spine in order to protect itself from becoming extinct during ages past. This hypothesis is purely conjectural. The large number of cactus species of the present day are all of the spiny type and the spineless species described are very few. For want of authentic records, it is, therefore, difficult to state whether spineless cactus arose as a mutation from the spiny type or *vice versa*.

"Reversion," "throw back" or "atavism" of characters is a phenomenon which is accepted by geneticists to indicate a reversion to the old or ancestral form. This phenomena affords a clue to the history of a character. In the light of this the reverse mutation which forms the subject of this note shows that the spiny character in cactus is primitive or ancestral compared to the spineless character.

The species to which the spineless cactus, grown in the Economic Botanist's Area in Poona (which has thrown off two spiny mutants), belongs is described by Burns (1940) as *Opuntia decumana* while Mehta (1923) includes it in *Opuntia ficus-indica*. The spineless plants have been under the constant observation of the writer for the past ten years, and until two years back no spiny type was seen to arise from the spineless type. Two years

FIG. 1.—The joints at the bottom are spineless. The middle joint on edge view has spines on the surface to the right while the surface to the left is spineless.

back, however, in a trial undertaken to compare the effectiveness of spineless cactus as a live-hedge, one set or joint out of several hundreds of the spineless type that were planted gave rise to spiny and spineless joints subsequently. The photographs illustrate the type of somatic mutation which gave rise to the joints bearing spines.

A single spineless joint (phylloclade) was planted with one-third of its portion buried underground. From this several subsidiary spineless joints were produced and from one such arose a joint which had spines on one of its flat surface while the opposite surface was spineless (Fig. 1). This is evidently a case of sectorial chimera which has arisen due to somatic mutation. Later, from this joint showing spine on one side and none on the other arose another joint which likewise repeated the characteristics. In the second joint, however, some spines are to be seen along the margin of the spineless surface.

The other instance (Fig. 2) arose independent of the above in a plot where joints of spineless cactus were planted for multiplication. From this arose joints without spine and one having spines. The spiny type subsequently gave rise to joints bearing spines.

In the second example it is noticed that the few segments which bore spines profusely are giving rise to subsequent joints with fewer and fewer spines. This is an interesting example of successive reversions taking place in the course of two years.

The above two instances very clearly illustrate the sudden origin of a spiny cactus from the spineless type by somatic mutation of a bud from which the joints arose. On the hypothesis of reversion to ancestral or primitive character, it would appear that the cactus types having spines have had an earlier origin. If this is considered with Willis's age and area hypothesis, the extensive areas over which the cactus (spiny) has spread in its own home in South America and the greater number of species as represented by it would definitely indicate that the spineless cactus is of more recent origin.

FIG. 2.—From a spineless joint, spiny joints have arisen to the right and spineless to the left. Of the spiny joints on the right, those below are completely covered with spines, while those towards the top show decrease in the number of spines.

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College of Agriculture,
Poona,
July 6, 1942.

[1] Burns, W., *Indian Farming*, 1940, **1**, No. 4, 160.
[2] Burbank, Luther, *A Partner of Nature*, Appleton Century Inc., 1939.
[3] Mehta, H. G., *The Agri. Jour. of India*, 1923, **18**.
[4] Willis, J. C., *Age and Area*, Cambridge University Press, 1922.

REVIEWS

Radiology Physics. By John Kellock Robertson. (Chapman and Hall, Ltd., London), 1941. Pp. 270. Price 18*s.* net.

The ever-increasing applications of physics in medicine make it imperative that the medical student should have a working knowledge of certain branches of physics. This knowledge would be particularly useful if he later specialises in radiology. The book under review, by Professor Robertson who is well known for his *Introduction to Physical Optics*, provides a suitable medium for the acquisition of such knowledge in branches of physics useful to the radiologist.

The first three chapters deal with an elementary treatment of alternating current theory and the production, control and measurement of high voltages. All that the radiologist need know about cathode rays, positive rays and X-rays find a place in the next five chapters. Chapter IX is devoted to an elementary treatment of diffraction of light, spectra, infrared and ultraviolet radiations and their uses in medicine. Chapter X is about measurement of X-ray wavelengths and the simple theory of the origin of characteristic X-rays. Chapters XI and XII deal with X-ray absorption, production of secondary radiation, the principle of the Potter Bucky diaphragm and measurement of X-ray dosage while Chapter XIII is devoted to redioactivity. The next two chapters are of special interest as they are devoted to recent developments on the production and use of high speed particles. Brief and readable descriptions of the cascade generator, the million volt X-ray tube, the Van de Graaf electrostatic generator and the cyclotron form the subject-matter of Chapter XIV. This is followed up, in the next chapter, on artificial radioactivity, by a brief resume of the applications of nuclear physics to medical and biological problems. Most of the work done on these lines is of recent origin and this makes the book under review all the more valuable. The final chapter of the book is devoted to the production and use of high frequency-currents. The book may be recommended for the use of pre-medical students and radiologists. It is also suitable for general study by physics students of B.Sc. (pass) classes. S. R. S.

Annual Review of Biochemistry, Vol. X. By J. M. Luck and J. H. C. Smith. (Annual Reviews, Inc., Stanford University P.O., California), 1941. Pp. xi + 692. Price $5.00.

The present review has been published in the second year of war which has extended to yet another European nation. The progress of scientific research, particularly its fundamental aspects, has received a regrettable but inevitable setback in most of the countries affected by the scourge of war. But a preliminary survey of the contents of the volume do not reveal any perceptible slacking of scientific effort during the year. It will, however, be noticed that of the twenty-four subjects discussed in the volume, no less than twenty-three have emanated from the various laboratories in America. This circumstance will not take away from the volume the international character which has constituted a special feature of these Annual Reviews.

The generous extension of American hospitality to European scholars who felt compelled to quit their fatherland, has, by introducing the international element, enriched American science; this is already reflected in the present volume under review.

In reviewing, in the field of Biological Oxidations and Reductions E. S. Guzman Barron refers to electromotively active systems—metalloporphyrins, flavoproteins and the more recently studied echinochromes. Valuable data regarding the oxidation-reduction potentials has been given and their significance in relation to cell respiration discussed. Reference has been made to activating proteins, whose isolation, purification and characterisation are proceeding slowly but steadily. Metalloprotein enzyme systems which include the various oxidases, catalase and carbonic anhydrase are referred.

The remarkable discovery of diphosphothiamin as the coenzyme of carboxylase has stimulated a considerable amount of work on the mechanism of the physiological role played by thiamin. The suggestion that diphosphothiamin might catalyse all the reactions where carbon dioxide acts as the

oxidising agent, opens up one of the most fruitful and spectacular fields of research.

Proteolytic enzymes are discussed by Bergman and his associate, while the non-proteolytic enzymes is reviewed by Tauber. Valuable reviews on the metabolism of carbohydrates, fats and proteins and amino acids appear in the present volume. Special attention should be invited to the reviews on Detoxication mechanisms and Harmones. Dieticians and pediatricians will find the review on Nutrition, illuminating and instructive. Reviews on soil deficiencies and animal nutrition and spectrometric studies in relation to biology, appear for the first time in this series. The latter review is presented in a form so as to be useful to those who wish to interpret spectroscopic data.

Barker's review on the chemistry and metabolism of bacteria discusses the nutrient and vitaminic requirements of micro-organisms and gives a survey of the work subsequently stimulated by the remarkable discovery of Wood and Workman regarding the utilisation of carbon dioxide. Other reviews relate to nitrogen fixation, protein monolayers, mineral nutrition of plants, plant-growth substances, bioluminiscence, water- and fat-soluble vitamins, biochemistry of nucleic acids, purines, pyrimidines, creatine and creatinine, sulphur compounds and carbohydrates and glycosides. The usual high standard has been maintained and the principal object of these reviews, a critical appraisement of the year's work, and a speculative but promising forecast of the future, has been more than amply fulfilled. We fervently wish that the present conflict will not in any manner affect the publication of this Annual which has come to be regarded as a landmark in the progress of Biochemistry.

Mechanism and Chemical Kinetics of Organic Reactions in Liquid Systems. A general discussion arranged by the Faraday Society. (Messrs. Gurney & Jackson, Ltd., London), 1941. Pp. 601 to 806. Price 15*sh.* 6*d.*

The book is a collection of papers contributed to a discussion in September last. Soon after the *Transactions of the Society* for December containing these papers was received in this country, a brief critical review was published in *Current Science* (Vol. 11, p. 202).

In 1937, the Faraday Society held a discussion on Reaction Kinetics in general and the present publication is a logical sequel. The earlier work dealt largely with the theoretical treatment of activation energy and reaction velocity, while the present one is naturally concerned with the application of these ideas. The fourteen papers that have been presented clearly indicate a unity of purpose, an application of exact technique together with physical understanding. The series of papers also draws attention to the necessity of a theory that envisages both the collision and the transition state methods of interpreting chemical reactions.

The publication is an illuminating work suggestive of further work in a rich field and should find a place in the library of every chemist.

Carnegie Institution of Washington: Year-Book No. 40, 1940-41. (Carnegie Institution of Washington, Washington, D.C.), 1941. Pp. xxxii + 346. Price $1.00 paper cover, $1.50 cloth binding.

The Carnegie Institution of Washington, which "occupies a unique and important place in the scientific affairs" of the United States of America, is dedicated to "the extension of man's fundamental knowledge of his environment". The report of the President which prefaces the Year-Book, outlines the way in which the scientific activities of the Institution have been oriented to meet the situation imposed on the Nation by war. "Events of the past two years have profoundly altered the plans and outlook of every scientific institution in the world, and of the great majority of individual scientists." Many of the long-range programmes of research in the field of pure science have now been changed or held in abeyance.

In discussing the function of scientific institutions in relation to the programme of defence the President writes: "There is not complete unanimity in this country as to how, or when, or to what extent the power of the nation should be exerted to defend our way of life. There is substantial unanimity, however, on the thesis that the power of the nation should be increased as rapidly as possible and to the maximum possible extent. Here the Institution has a very

definite duty. Military strength has been definitely demonstrated to depend, in no inconsiderable degree, upon the intelligent application of science to military devices and operations. As a great and unique scientific organisation of national extent, with its central offices close to the seat of Government, the Institution has a duty far beyond that of responding passively to the calls of Government for the loan of the services of members of its staff. It is called upon to participate actively, in co-operation with other scientific groups, in bringing to the aid of Government the co-ordinated intense effort of the scientists of this country, supplementing the activities of the armed services, in order that the weapons placed in the hands of the youth of the land may be fully adequate. The scientists of the Institution are discharging this duty to the full extent of their ability and opportunity."

The Carnegie Corporation of New York, through whose munificence the scientific activities of the Institution are kept alive, has recognized that the continued maintenance of the prominence of an Institution, involves the intensification of efforts in new directions and new approaches to old problems by the adoption of new methods offered by modern instrumentation. In pursuance of this progressive and enlightened policy, the Institution has installed a large cyclotron to attack the many borderland problems between physics and biology. A study has also been undertaken toward a new approach to human genetics.

While a substantial portion of the resources of the Institution are now harnessed for prosecuting the defence programmes, the administration has not overlooked the importance of keeping the fountain of fundamental research flowing. The President remarks: "Fundamental scientific research is almost completely stopped all over the world, except in this hemisphere. The inspiration passed from master to disciple, and the subtle evolution of great ideas when powerful minds collaborate, or compete, are part and parcel of the rapid progress of modern science. This implies continuity of effort. If the thread is broken it may be long before it can be mended. With science and scientists in other lands completely distracted by immediate requirements, an organization such as ours has a responsibility for preserving some of the more important threads in tact. This duty has not been forgotten, although its fulfilment becomes increasingly difficult." M. S.

CENTENARIES

Wright, Benjamin (1770–1842)

BENJAMIN WRIGHT, known as the father of American Engineering, was born in Wethersfield, Connecticut, October 10, 1770. Having a talent for mathematics, he studied surveying and he persuaded his father who was a petty farmer to move into the new settlements of New York and carried out land surveys of over 500,000 acres between 1792 and 1796.

As the area developed Wright interested himself in improving its transport facilities by constructing canals. The experience which he thus built up got for him a prominent place in the construction of the Erie Canal in 1817 which he completed in 1825. In executing the work, he gathered around him a remarkable group of young men all of whom afterwards developed into engineers of first quality and thus earned for Wright the familiar appellation 'Father of American Engineering'.

Wright died in New York City, August 24, 1842.

Reynolds, Osborne (1842–1912)

OSBORNE REYNOLDS, a British physicist, was born at Belfast, August 23, 1842. His father who was fourth wrangler and a school master, paid personal attention to his son's education and had admitted him into the workshop of a mechanical engineer before he entered the Queen's College, Cambridge. He graduated in 1867 as the seventh wrangler and became the first professor of engineering in the Owen's College, Manchester. This post he held till his retirement in 1905.

During his long tenure of professorship Reynolds made many investigations most of which sought to find mechanical explanations of physical phenomena like lubrication, flow of water in pipes and the concept of critical velocity, dilatancy of granular media, and group velocity of waves. The most extensive piece of experimental work he carried out was the determination of the mechanical equivalent of heat by the direct measurement of the amount of heat required to raise a pound of water from the freezing point to the boiling point.

Reynolds became a fellow of the Royal Society in 1877 and got its gold medal in 1888. His *Papers on mechanical and physical subjects* were published in three volumes in 1900-03.

Reynolds died in Somerset, February 21, 1912.

S. R. RANGANATHAN,
University Library,
Madras.

SCIENCE NOTES AND NEWS

Vegetable Insecticides in India.—The discovery of several useful vegetable insecticides and the possibilities of their cultivation in India are announced by the Forest Research Institute, Dehra Dun.

For controlling agricultural as well as household pests vegetable insecticides are preferred to others, such as, lead and copper salts, arsenic and nicotine, because they are non-poisonous to man and animals.

The growing demand for vegetable insecticides was hitherto met mainly by a plant called "derris" from Malaya, Dutch East Indies and Philippines. Investigations conducted by the Forest Research Institute have now shown that other plants bearing the same toxic content as "derris" are available in this country; and the existing material is rich enough for the preparation of effective insecticidal emulsions and powders. Their toxic content is capable of still further improvement by proper cultivation and treatment.

Certain parts of India, it has been found, possess suitable climatic and soil conditions for the introduction and cultivation of richer varieties of Malayan "derris". Experiments in this direction have already proved successful in Mysore, Cochin and Assam.

Alcoholism and Thiamin.—Heavy drinkers of whisky and other alcoholic beverages probably do not require extra amounts of vitamin B_1 to protect their nerves and keep them healthy. Experiments casting "considerable doubt" on the current theory that alcohol increases the body's need for this vitamin were reported by Dr. J. V. Lowry, Dr. W. G. Sebrell, Dr. F. S. Daft and Dr. L. L. Ashburn, of the U.S. National Institute of Health. In these experiments rats kept on the water wagon without exception developed the severe nervous disorder believed due to B_1 deficiency in alcoholism before their litter mates that were getting alcohol or whisky. The nervous disorder could be prevented and cured by the vitamin, regardless of whether the rats drank alcohol, water or whisky. These experiments give the first indication that alcohol does not require vitamin B_1 to help burn it in the body. They suggest that a person who sticks to a good diet could probably drink a quart of whisky daily without needing extra vitamin B_1 to burn the alcohol. If, however, he neglects his diet, as alcoholics probably do, and fails to eat enough food containing vitamin B_1, he would develop the nervous disorder. The whisky or alcohol could be blamed for causing the sickness by depleting the body of the vitamin.

Science, 95, 2467, p. 8 (Supp.)

The Indian Jute Industry and the American Bagging Problem.—According to the July issue of the Indian Central Jute Committee *Bulletin*, exports of raw jute from India during the eight months from July 1941 to February 1942 were 205,000 tons as against 142,700 tons and 387,500 tons during the corresponding periods of the 1940-41 and 1939-40 seasons respectively. The total volume of raw jute exports during the first three months of the Pacific War was reduced by more than one-fifth of the volume in the same period of the previous year. Exports of jute manufactures during the first eight months of the jute-year 1941-42 were 636,500 tons as compared with 581,400 tons and 762,700 tons in 1940-41 and 1939-40 respectively. The total jute trade during the period declined but not to the same extent as in the previous year.

The American Republics need bags in connection with a large percentage of their Agricultural Crops. Some idea of the importance of Indian jute to these countries may be gathered from the available import and consumption statistics. In 1940, Chile imported 6,000,000 jute sacks and produced an additional 3,000,000 from imported jute fibre. Argentine uses about 378,000,000 sacks a year. Brazil purchased about 48,000,000 lbs. of jute in 1940 and Peru about 25,000,000 lbs. Chile will require 24,000,000 sacks during 1942.

With the growing difficulties in the jute shipments and the dependence of America on jute bagging, there has been, of late, considerable experimentations for substitutes. Cotton bags and multi-wall paper bags are rapidly gaining the position lost by jute.

A synthetic jute factory is being established at Foxton, near Wellington, New Zealand, to manufacture synthetic jute for felt production, utilising wool-pack waste together with odd ends and pieces of cloth. In this connection it is gratifying to note that the Indian Central Jute Committee at its meeting held on July 25, 1942, approved of the extension of the programme of work of the Technological Research Laboratories of the Committee, Mr. P. M. Kharegat, C.I.E., I.C.S., presiding.

Although the Laboratories at present do not possess the necessary equipment for a full-scale work of this nature, the Committee decided to make an immediate start with the existing facilities, and to recommend to the Central Government for early sanction of necessary finance for some additional facilities.

The general nature of work proposed to be done in this connection will be, first to see to what extent jute can be used to replace fibres, such as flax and Italian hemp, which are now more or less unavailable in India; and, secondly, the production of ply threads of the greatest possible strength, regularity and durability.

Such work is also likely at the same time to be of value in connection with discovery of new or extended uses of jute.

A New Blackout Bulb.—A blackout bulb, writes Edwin Neff, in *Science*, 95, 2466, which eliminates need for special drapes and shades, gives ample light to avoid stumbling over

furniture, yet cannot be seen from the air has been successfully developed and tested by Army engineers at Fort Belvoir, Va., and will probably soon be on the market. The new bulb is heavily coated with black except for an orange button about the size of a nickle on the bottom. It burns on average house current and will sell for about 25 cents. One bulb per room will provide enough light to permit occupants to see each other plainly, as well as furniture, doors and windows. Only the usual household curtains, drapes or shades are needed when this bulb is the sole source of light. Army pilots and engineers tested the bulb recently in a tiny town in New Jersey (only forty houses). Each home was equipped with the blackout bulbs and shades and curtains left up. When pilots flew over they were unable to see a single ray of light. The bulbs were developed with the co-operation of the Nela Park Engineering Department of the General Electric Company at Cleveland, Ohio. Army engineers explained that orange was selected as the colour for light-emitting button, since it is near the red end of the spectrum, yet unlike red is not confused with exit lights. Red has been found to be the light least visible from the air.

While the blackout bulb will not permit reading or playing at cards, it is safer and more convenient than no light at all. It can be used to light sections of the house where there are too many doors or windows for the practical use of blackout drapes. One room of the house can be blacked out completely to permit reading with ordinary light, while the rest of the house can be lighted with the special bulbs.

Reclamation of Agar.—Hilda G. Macmorine describes a method (*Can. Public Health J.*, 1942, 23, 39) for reclamation of agar from used culture media. The agar is melted by autoclaving and poured into cylinders. Media in petri-dishes heavily infected or containing blood or serum are placed into boiling water. The melted agar filtered through cheese cloth to remove the coagulated blood and serum and the bulk agar put into cylinders as before.

After the agar sets, it is removed, cut into thin slices and washed well with cold water free from broth. The washed agar is remelted, decolourised with carbon black and filtered through the Buchner funnel lined with filter-paper and paper pulp.

The resulting clear agar solution is autoclaved for 1½ hours at 250° F., with potassium oxalate and potassium carbonate. The hot agar is then filtered and poured into two volumes of cold acetone with vigorous stirring. The agar comes down in fairly granular form, which is filtered in a Buchner funnel, washed with acetone and dried. Ethyl alcohol may be used for precipitating the agar, but the precipitate is rather colloidal and difficult to separate by filtration.

This modified agar gives a hardness, only slightly inferior and is in no way bactericidal.

V. S. G.

Ergot Cultivation.—The ergot fungus (*Claviceps purpurea*) is parasitic on various grasses particularly rye grains transforming them to hard sclerotia. Infection takes place at the flowering period. The sclerotium is rich in ergotoxine which is highly valued in medicine. The conidial or *sphacelia* can be cultured on synthetic media and stored at low temperatures (Melville, R., in *Pharm. J.*, 1941, p. 178) when the glumes of rye flowers open and the anthers are exserted out, they can be infected with the conidiospores. By following this method, ergot has been cultivated on a large scale in Europe.

In India *Sphacelia sorghi* has been known to occur on *Sorghum vulgare*, *Andropogan caricorus*, *Ischæmum*, *Brachypodium* and other grasses (*Cur. Sci.*, Nov. 1941). Formation of sclerotia takes place, but the perfect stage has not been observed. If culturing of *Sphacelia* occurring in India be started, ergot cultivation can be attempted on a large scale. M. J. T.

Erosion in Cultivated Uplands.—The Punjab has led other provinces in India in pioneering anti-erosion measures on a large scale; indeed, this has developed into one of the main activities of the Punjab Forest Department which in collaboration with the Revenue, Agricultural and Co-operative Departments, have done much to arouse and educate the public into these problems. In a Bulletin entitled "Erosion in the Cultivated Uplands of the North Punjab and Its Cure" (Government Press, Lahore, 1941), Mr. H. M. Glover, I.F.S., records the success of the Co-operative Land Reclamation Societies in carrying out anti-erosion measures. No details are given but the Bulletin includes very telling photographs, some of these taken from air. In a Foreword, Mr. F. L. Brayne makes the point that in the North Punjab, "the land aches for labour" and that if tangible results are to be obtained "every one shall work together" and that the best results are obtained when such co-operative endeavour is "scientific". Mr. Glover's Bulletin tells in simple language how the Forest Department is ready and eager to assist with such scientific advice any one who cares to apply.

Wood preservation is a highly technical job and the average layman is not well placed in choosing a process which is efficient for his particular needs; he is apt to overlook the fact that the ideal wood preservative is yet to be discovered; that each process has its own merits and limitations. And the high sounding, contradictory and sometimes extravagant claims made for the proprietary preservatives tend to confuse him still further. It is to educate such prospective users that "A Short Note on Wood Preservation for Users in India" by Dr. D. Narayanamurthy has been published as *Indian Forest Bulletin* No. 110 (Manager of Publications, Delhi, 1942. Price As. 12 or 1*sh.*). A broad outline is given, in non-technical language, of wood preservatives, the processes of treatment, costs and the handling of timber subsequent to treatment. The text is illustrated with four good photographs and a figure with lettering none too easy to decipher.

Under "Other Treating Processes", "Powelling" (which makes use of molasses as the preservative base) is a notable omission. It would have considerably added to the value of the publication to the layman if some authentic "service records" of treated timber (as for example those maintained and published by the American Wood Preservers' Association) had been included; such data although not always applicable to Indian conditions would still be indicative of the comparative "life" one could expect for timber treated with the different preservatives.

Indian Timber for Aircraft and Gliders.—With India undertaking aircraft and glider construction, interest in Indian woods likely to be of use for this type of work has been intensified.

The Forest Research Institute, Dehra Dun, which has investigated the subject and organised tests for compiling comparative information on the strengths of Indian woods, has just published a "Note on Indian Timbers for Aircraft and Gliders". According to this publication, Indian spruce of aircraft standard is obtainable in lengths up to possibly 10 or 12 feet, but the larger lengths of clear timber are not likely to be available. Small quantities of Indian aircraft spruce have already been extracted and put into use. Arrangements are now in train to organise the supply of larger quantities, and a forest officer has been placed on special duty to organise continued supplies of specially selected spruce and fir for aircraft work in India.

The wood mainly used for aircraft work in other countries is Sitka spruce, a tree of the western Canadian and American forests, which is not indigenous in India. But India also has a spruce species, and if properly selected wood is used, it compares favourably in strength with Sitka spruce. The Indian species is, however, a very knotty wood, and it is difficult to obtain clear aircraft material in long lengths to meet all the requirements. Even with Sitka spruce only 5 to 10 per cent. of the timber cut is suitable for aircraft work, but good lengths of clear timber free of knots can be obtained with that species.

The note emphasises the need for proper selection and gives specifications for the selection of Indian spruce for aircraft work. It also suggests that one or two of the lighter Indian hardwoods, such as *champ* or *bonsum*, may possibly fill the deficiency where longer lengths of aircraft timber are required.

Brief descriptions of several Indian species considered suitable for making aircraft plywood and propellers are also given.

The Occurrence of the Nematode Genus *Oswaldocruzia* in India.—The genus *Oswaldocruzia* (Family Trichostrongylidæ) was erected by Travassos in 1917. Quite a large number of species of this Genus have subsequently been described from Amphibian hosts in various countries; but so far as I am aware this genus has not hitherto been reported from India. Baylis and Daubney described one species from Nicobar Islands in 1923. While collecting Nematodes from various Amphibian hosts, I obtained a few specimens of *Oswaldocruzia* from the intestine of the common toad *Bufo melanostictus* at Lucknow. This would thus be the first record of the Genus in India. The present form affords interesting material for study and appears to be new to science. A detailed account of its morphology and toxonomy will shortly be published. M. B. LAL.

Working of the War Resources Committee.—In a communique the Government of India announce:—The President of the War Resources Committee is His Excellency the Viceroy and his Deputy is the Honourable Sir Homi Modi, Supply Member, by whom the chair is occupied in the ordinary course.

The Honourable Members for Commerce, Communications and Finance, form the body of the Committee and a representative of the Commander-in-Chief, who is constitutionally a Member of the Committee, also attends. It is not yet known whether the recent expansion of the Viceroy's Executive Council will result in any changes in the composition of the Committee.

The Committee deals with matters arising out of the Grady Report and those initiated by the various Departments or by the Chairman and Secretariat of the Committee. Where departments other than those already represented in the Committee are affected the Honourable Member concerned is invited to attend or send a representative.

Decisions of the Committee carry the full authority of the Executive Council and the departments concerned see to their execution. Progress Reports are usually asked for by the Committee in order that it may keep in touch with developments.

The Secretary of the Committee is Lt.-General T. J. Hutton, till recently G.O.C. in Burma and formerly Chief of the General Staff, India, and it is understood that Mr. T. M. S. Mani, I.C.S., of the Communications Department, will shortly assume the appointment of Deputy Secretary.

University of Calcutta.—Mr. S. R. Palit, M.Sc., of the Indian Lac Research Institute, Ranchi, has been admitted to the Degree of Doctor of Science of the University of Calcutta on a thesis entitled, "Fundamental Physicochemical Investigations of Solutions of Resins, Cellulose derivatives and Soaps".

SEISMOLOGICAL NOTES

During the month of July 1942 five moderate and three slight earthquake shocks were recorded by the Colaba seismographs as against seven slight ones recorded during the same month in 1941. Details for July 1942 are given in the following table:—

Date	Intensity of the shock	Time of origin I. S. T.	Epicentral distance from Bombay	Co-ordinates of the epicentre (tentative)	Depth of focus	Remarks
		H. M.	(Miles)			
3	Moderate	08 20	620	Near Lat. 25°N., Long. 65°E., in Baluchistan	..	Felt in Karachi. Karachi experienced two earthquake shocks of moderate intensity this morning at 8-36. It is reported that doors and windows rattled. No damage is, however, reported A.P.
4	Slight	14 17	630	..	..	Probably an aftershock of the previous one.
8	Moderate	12 26	10110	..	..	
10	Slight	08 51	1570	..	..	
12	Moderate	10 35	10150	..	..	
24	Slight	10 30	2350	..	..	
25	Moderate	11 53	3570	..	125 km. (Probable)	
30	Moderate	04 19	4030	Lat. 4°S., Long. 129° E., near the Island of Ceram in Banda Sea.	80 km.	

MAGNETIC NOTES

July 1942 was more disturbed than the preceding month. There were 14 *quiet* days, 16 days of *slight* disturbance and one of moderate disturbance as against 3 quiet days, 18 days of slight disturbance, 7 of moderate disturbance, 2 of great disturbance and one of very great disturbance during July of last year. The day of the largest disturbance during July 1942 was the 11th while the quietest day was the 4th. Characters for individual days were as follows:—

Quiet days	Disturbed days	
	Slight	Moderate
2-5, 9, 14, 18-19, 21, 22, 24, 29-31.	1, 6-8, 10, 12-13, 15-17, 20, 23, 25-28.	11.

No magnetic storm was recorded during July 1942 while two storms (one moderate and one very great) were recorded during July of last year. The mean character figure for July 1942 is 0·58 as against 1·23 for July 1941.

M. R. RANGASWAMI.

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"Journal of the Royal Society of Arts," Vol. 90, No. 4611.

"Journal of Agricultural Research," Vol. 64, No. 7.

"Agricultural Gazette of New South Wales," Vol. 53, Nos. 5 and 6.

"Annals of Biochemistry and Experimental Medicine," Vol. 2, No. 2.

"The Journal of Chemical Physics," Vol. 10, No. 4.

"Journal of the Indian Chemical Society," Vol. 19, Nos. 4-6.

"Experiment Station Record," Vol. 86, No. 4.

"Indian Forester," Vol. 68, No. 8.

"Indian Forest Records," Vol. 5, No. 1.

"Indian Farming," Vol. 3, No. 7.

"Quarterly Journal of the Geological, Mining and Metallurgical Society of India," Vol. 14, No. 1.

"The Indian Central Jute Committee," Vol. 5, No. 4.

"The Bulletin of the American Meteorological Society," Vol. 23, No. 2.

"Journal of the Indian Mathematical Society," Vol. 6, No. 1.

"Journal of Nutrition," Vol. 23, No. 4.

"Nature," Vol. 149, No. 3787.

"American Museum of Natural History," Vol. 46, No. 3; Vol. 47, No. 1; and Vol. 49, No. 3.

Books

"Mass Spectra and Isotopes," by F. W. Aston. (Edward Arnold & Co., London), 1942. Pp. xii + 276. Price 22*sh.* 6*d.*

"Plastics in Industry," by 'Plastes'. (Chapman & Hall, London), 1942. Pp. xiii + 248. Price 15*sh.*

"The Chemical Analysis of Ferrous Alloys and Foundry Materials, Modern Practice and Theory." (Chapman & Hall, London), 1942. Pp. xv + 362. Price 28*sh.*

"Mechanism and Chemical Kinetics of Organic Reactions in Liquid Systems," A General Discussion held by The Faraday Society. (Gurney & Jackson, London), 1941. Pp. 601 to 806. Price 15*sh.* net.

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